Investigation of lectin complement proteins in urinary schistosomiasis and visceral leishmaniasis

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät

der Eberhard Karls Universität Tübingen

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

vorgelegt von

Justin Antony Selvaraj

aus Pannirkulam, Indien

Tübingen

2015

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:	21.01.2016
Dekan:	Prof. Dr. Wolfgang Rosenstiel
1. Berichterstatter:	Prof. Dr. Peter G Kremsner
2. Berichterstatter:	Prof. Dr. Jürgen Tomiuk

TABLE OF CONTENTS

LIST OF PUBLICATIONS

This thesis is based on the following publications.

(*Shared first author)

a) Published Papers

1. Antony JS, Ojurongbe O, Tong HV, Ouf EA, Engleitner T, Akindele AA, Sina-Agbaje OR, Adeyeba AO, Kremsner PG, Velavan TP: **Mannose-binding lectin and susceptibility to schistosomiasis**. *J Infect Dis* **2013**, 207(11):1675-1683.
2. Antony JS, Ojurongbe O, Kremsner PG, Velavan TP: **Lectin complement protein Collectin 11 (CL-K1) and susceptibility to urinary schistosomiasis.** *PLoS Negl Trop Dis* **2015**, 9(3):e0003647.
3. Antony JS, Ojurongbe O, Meyer CG, Thangaraj K, Mishra A, Kremsner PG, Velavan TP: **Correlation of Interleukin-6 levels and lectins during *Schistosoma haematobium* infection.** *Cytokine* **2015**. doi:10.1016/j.cyto.2015.04.019
4. Mishra A, Antony JS*, Sundaravadivel P, Tong HV, Meyer CG, Jalli RD, Velavan TP, Thangaraj K: **Association of Ficolin-2 serum levels and *FCN2* genetic variants with Indian visceral leishmaniasis.** *PLoS One* **2015**, 10(5):e0125940.
5. Ojurongbe O, Antony JS*, Tong HV, Meyer CG, Akindele AA, Sina-Agbaje OR, Kremsner PG, Velavan TP: **Low MBL-associated serine protease 2 (MASP-2) levels correlate with urogenital schistosomiasis in Nigerian children**. *Trop Med Int Health* **2015**, 20(10):1311-1319.
6. Mishra A, Antony JS*, Gai P, Sundaravadivel P, Tong HV, Jha AN, Singh L, Velavan TP, Thangaraj K. **Mannose-binding lectin as a susceptible factor influencing Indian Visceral Leishmaniasis.** *Parasitol Int* 2015, 64(6):591-596.

PERSONAL CONTRIBUTIONS

My contributions to the papers include:

Manuscript 1:

- DNA isolation, Genotyping of six functional variants by direct sequencing
- Data analysis

Manuscript 2:

- Conceived and designed the experiments
- Genotyping of five functional variants in *COLEC11* by direct sequencing
- CL-K1 protein level measurement by ELISA
- Data analysis, writing of the manuscript

Manuscript 3:

- Conceived and designed the experiments
- IL-6 serum level measurement by ELISA
- Data analysis, writing of the manuscript

Manuscript 4:

- Ficolin-2 protein level measurement by ELISA
- Data analysis
- Writing of the manuscript

Manuscript 5:

- Genotyping of nine functional variants in *MASP2* by direct sequencing
- MASP2 protein level measurement by ELISA
- Data analysis, writing of the manuscript

Manuscript 6:

- MBL protein level measurement by ELISA
- Data analysis
- Writing of the manuscript

SUMMARY

Complement lectins are pathogen recognition receptors (PRRs) that bind to pathogen associated molecular patterns (PAMPs) of various microbes. The circulating serum levels and functional genetic variants of four such innate immune recognition elements, namely the human mannose-binding lectin (MBL), ficolin-2 (FCN2), collectin 11 (CL-K1), mannose-binding associated serine protease-2 (MASP2) were studied in intracellular (visceral leishmaniasis) and extracellular (urinary schistosomiasis) parasitic diseases. In extracellular *Schistosoma haematobium* infection, MBL, MASP2, and collectin-11 (CL-K1) and their functional variants were associated with relative protection. In intra-cellular *Leishmania donovani* infection, MBL, ficolin-2 and their functional variants were observed to be a susceptible host factor. IL-6 was observed to regulate the lectin expression during distinct parasitic infections. In conclusion, this dissertation provides probable evidence on the differential role of lectins in intra and extracellular infections.

ZUSAMMENFASSUNG

Komplement-Lektine sind sogenannte “pathogen recognition receptors” (PRRs), welche Pathogen-assoziierte molekulare Muster (“pathogen associated molecular patterns”, PAMPs) diverser Krankheitserreger erkennen. Ich untersuchte zirkulierenden Serumlevel und funktionelle genetische Varianten von vier solcher PRRs in einer intrazellulären (viszerale Leishmaniose) und einer extrazellulären (urogenitale Schistosomiase) Krankheit: Mannose-bindendes Lektin (MBL), Ficolin 2 (FCN2), Collektin 11 (CL-K1), und MBL assoziierte Serinprotease 2 (MASP2). In der extrazellulären *Schistosoma haematobium* Infektion waren MBL, MASP2, und Collektin 11, sowie deren funktionelle Varianten mit einem relativen Schutz gegen die Krankheit assoziiert. In der intrazellulären *Leishmania donovani* Infektion waren MBL, Ficolin 2, und deren funktionelle Varianten Suszeptibilitätsfaktoren. Während distinkter parasitischer Infektionen regulierte IL-6 die Lektinexpression. Zusammenfassend erbringt diese Dissertation diverse Beweise für die differentielle Rolle von Lektinen in intra- und extrazellulären parasitischen Infektionen.

GENERAL INTRODUCTION

1. Human Immune System and Infection

A host can evolve two types of defense mechanisms to ensure its survival when challenged by a pathogen: resistance and tolerance [1]. The human immune system resists the invading pathogens. The immune system is broadly divided into innate and adaptive immunity, but, both are interconnected and aid in elimination of pathogens [2]. The adaptive immune system consists of T and B lymphocytes, which functions as antigen-specific recognition to identify and eliminate the pathogen and ensures a long-lived immunological memory against reinfections [3].

1.1 Innate Immunity

Innate immunity is a first line of the host's defense against infections. It provides robust, immediate and non-specific immune responses to invading pathogens. Innate immunity is further divided into evolutionarily primitive humoral and cellular components. [4]. Innate immune system consists of anatomical barriers, complement system and natural killer cells, phagocytes, pattern recognition receptors (PRRs), toll-like receptors (TLRs) that play a vital role in the protection of the host from pathogenic challenges [5].

1.2 Complement System

The complement system was first identified as a heat-sensitive factor in fresh serum for its ability to 'complement' the antibacterial properties of antibody [6]. The complement system can be activated through three major pathways: classical, lectin, and alternative. The complement system consists of 30-40 plasma and cell membrane proteins and functions between the innate and adaptive immune systems. It plays a vital role in host defenses against infections. Activation of the complement leads to robust and efficient proteolytic cascades to eliminate pathogens by inducing chemotaxis (attraction of leukocytes), opsonophagocytosis, and direct destruction of the microorganisms by enabling membrane attack complexes (MAC) in the cell wall [7].

2. Lectin Complement Pathway

The lectin complement pathway plays a key role in innate immunity by recognizing invading pathogens [8]. The lectin pathway is initiated by the binding of mannose-binding lectin (MBL) and other c-type lectins to sugar moieties exhibited on cell surfaces of several microbes and activates the esterase activity of MBL-associated serine proteases (MASP-1,2 and 3). Once activated, MASPs cleave and activate C4 and C2 thus generating the C3 convertase (C4bC2a). C3 convertase binds and cleave C3, generating C3b and C3a. The C3a fragment has anaphylatoxic and proinflammatory activity where as C3b has opsonic activity. C3b is also involved in C5 convertase (C4b2a3b) generation and cleaves C5, C6, C7, C8, and C9 proteins, resulting in the assembly of membrane attack complexes (C5b-9) on the target pathogen [9]. Unlike the classical pathway, the activation of the lectin pathway is independent of a specific antibody response. The early activation of the complement system during pathogen invasion occurs mainly by lectin pathway with a rapid response, since it is independent of specific antibody response. The lectin pathway also triggers the activation of the alternative pathway by generating C3b, which results in synergistic activation of the complement system [10]. Due to its essential role in innate immunity, the lectin pathway has been preserved throughout animal and hominoid evolution [11]. Lectin deficiency is associated with susceptibility to infections [12].

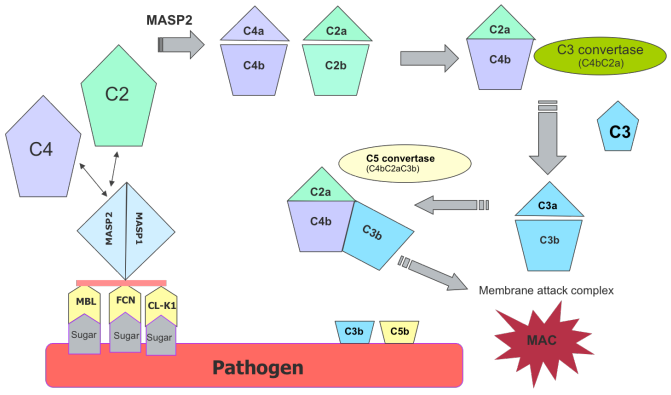

Figure 1: Overview of the lectin pathway of complement activation. MBL, CL-K1 or ficolins interact with pathogen's glycoproteins and initiate the complement cascade that result in pathogen lysis. Figure courtesy to Dr. Velavan.

Mannose-Binding Lectin (MBL)

MBL was the first lectin protein described to initiate the lectin pathway. It is synthesized in the liver and acts as an acute-phase plasma protein (APP) during infections. MBL is an oligomer consisting of three polypeptide chains, each containing a collagen-like domain and a carbohydrate recognition domain (CRD) similar to C1q to recognize pathogens [13]. MBL deficiency (low serum level) affects around 30% of the human population and is associated with disease susceptibility [14]. MBL encoded by the *MBL2* gene on human chromosome 10q11.2-10q21 [15]. *MBL2* gene polymorphisms are associated with circulating MBL serum level and functional activity. In specific, three promoter variants localized at positions − 550(*H/L*), − 221(*X/Y*) and + 4(*P/Q*) modulate the MBL transcription. In addition, three non-synonymous variants in exon-1 at codons 52 (*MBL2*D*), 54 (*MBL2*B*) and 57 (*MBL2*C*) disrupt the sequence of Gly-Xaa-Yaa tandem repeats in collagen-like domain and results in impaired oligomerization of the MBL protein [16, 17]. Garred et al., reviewed that MBL deficient alleles have been found in different frequencies in different populations [18]. For example, in Quechua Amerindians from Peru, the *MBL2*B* allele has the frequency of 0.80 and almost replaced the wild type *MBL2*A* allele whereas *MBL2*C* allele is almost exclusively present in the African population at the frequency of 0.24 [19].

Due to the same chromosomal location, the combination these promoter and exon-1 variants resulted in seven common 'secretor haplotypes'. Among them the haplotypes *MBL2*HYPA*, *LYPA* and *LYQA* are associated with high expression of MBL, while the haplotypes *MBL2*LYQC*, *LXPA*, *HYPD* and *LYPB* are associated with low expression of MBL [17, 20]. Apart from these seven common secretor haplotypes, specific haplotypes have been found in few ethnic groups such as *LYPD* in Czech, *HXPA* in Morocco and *LYQB* in Cameroon [14]. MBL has been shown to bind to a wide range of microbes and activate the complement cascade [21]. The impact of MBL deficiency towards the susceptibility of various bacterial, viral, fungal and parasitic infections were described [22, 23]. Epidemiological studies have suggested that lower MBL serum level and MBL deficient alleles correlate with increased susceptibility to various infectious diseases [24].

Ficolins (FCN)

Ficolins are a group of oligomeric lectins and has a similar function that of MBL. In humans, three types of ficolins (Ficolin-1, Ficolin-2 and Ficolin-3) exist with similar structures, but differential tissue expression and functional roles [25]. Ficolin-1 (M-ficolin) is expressed in lung, spleen, monocytes and present in serum. Ficolin-2 (L-ficolin) synthesized in the liver and is present in serum whereas Ficolin-3 (H-ficolin) is expressed in liver and lung [26]. The Ficolin-1, -2 and -3 proteins are encoded by the *FCN1*, *FCN2* and *FCN3* genes respectively. The *FCN1* and *FCN2* genes are located in a tail-to-tail orientation on chromosome 9q34 whereas *FCN3* gene is located on chromosome 1p35. FCN consists of a collagen-like tail region and functional fibrinogen-like domain that recognizes the acetylated compounds of invading pathogens. Ficolin-1 and -2 bind to cellular components such as lipoteichoic acid and sialic acid that found in bacteria and activate complement cascade through MASP-2 [27].

Genetic variants in ficolin coding genes and their serum abnormalities have been investigated against many infectious diseases and has been established as a key component of innate immunity [28]. Number of polymorphisms that affect the expression and function of ficolins were listed in their corresponding coding genes. Though several polymorphisms have been identified in the *FCN1* and *FCN3* genes, the important functional variants have been reported in the *FCN2* gene. Three promoter variants in the *FCN2* gene at positions −986G>A, −602G>A, and −4A>G have been associated with reduced ficolin-2 serum levels. Two structural variants in exon-8 at positions +6359C>T (Thr236Met) and +6424G>T (Ala258Ser) were observed to show differential binding properties to acetylated compounds [23]. Similar to MBL, ficolins activate the complement cascade and induce opsonophagocytosis and stimulates the proinflammatory cytokines. Many investigations have been reported the role of ficolins in various infectious and autoimmune diseases [28]. Ficolin-2 deficiency has been associated with recurrent infections and low birth weight in neonates [29]. *Trypanosoma cruzi* calreticulin (TcCRT), a chaperone molecule protein inhibit the lectin complement cascade by interacting with ficolins-2 [30] signifies the importance of this lectin.

Collectin Kidney-1 (CL-K1)

CL-K1 (Collectin-11) is a recently discovered lectin pathway initiating protein and is ubiquitously expressed in many tissues, including the kidney, liver and adrenal glands [31]. Similar to MBL, CL-K1 has a collagen like domain and a Carbohydrate Recognition Domain (CRD) that binds to various bacteria, fungi and viruses [32]. The CL-K1 is encoded by *COLEC11* gene and is located on human chromosome 2p25.3. Structural genetic variants in *COLEC11* were associated with individuals affected with Carnevale, Mingarelli, Malpuech and Michels syndrome (also known as 3MC or Malpuech facial clefting syndrome), a congenital disorder characterized by facial clefting, a caudal appendage, growth deficiency and cognitive disabilities [33]. CL-K1 was observed to interact with MASP-2 in the presence of mannan and deposits C4b through complement activation [34]. Unlike MBL and ficolins, genetic association studies on CL-K1 with infectious diseases are inadequate.

MBL-associated serine protease-2 (MASP-2)

Upon recognition of carbohydrate structures on pathogens by lectins such as MBL, FCN and CL-K1, MASPs are activated to cleave the down-stream complement component C2 and C4 subsequently activates the lectin complement cascade [35]. MASP-2 is composed of six domains including a serine protease domain that exhibits the esterase activity. In humans MASP-1 (encoded by *MASP1* gene located on chromosome 3q27-8) and MASP-2 (encoded by *MASP2* gene located on chromosome 1p31.23-31) exist with similar structure and function. However, alternative splicing of the *MASP1* gene produces MASP-3. Among these, MASP-2 is the vital serine protease in the lectin complement activation that majorly expressed in liver [36]. Various naturally occurring polymorphisms including p.D120G and p.P126L in *MASP2* gene modulate the circulating MASP-2 levels, protease activity and binding affinity to MBL [37]. The MASP-2 serum levels and *MASP2* deficient alleles were observed to be diverse among different ethnicities [38]. MASP-2 deficiency is reported among Caucasians [39]. MASP-2 serum levels and their functional genetic variants were associated with several infectious diseases [40].

2.1 Dual role of lectins in distinct infections

Complement lectins deficiency *per se* increases the susceptibility to many infections. It was observed that lectins, in specific MBL play a dual role during intra and extra-cellular infections. MBL is a highly investigated serum lectin and considered as a "double-edged sword", as low MBL serum levels and MBL deficient alleles increases the susceptibility towards severe or recurrent infections caused by extracellular pathogens [41-43]. On the other hand, low MBL serum levels and MBL deficient alleles were shown to associate with protection against intracellular pathogens like *Mycobacterium spp.* and *Leishmania spp*. [44-46]. The hypothesis is that MBL deficiency may be protective against intracellular pathogens, as it enhances the uptake into macrophages, the milieu preferred by these pathogens for their survival. However, one meta-analysis of MBL with tuberculosis in different ethnicities and different *Mycobacterium* species have disproved the hypothesis [47].

Ficolins are other important lectin that analogous to MBL and expected to function similar to MBL against *Mycobacterium spp.* infection. In contrary, during *Mycobacterium tuberculosis* infection, the ficolin-2 serum levels were reduced in TB patients compared to healthy controls [48]. Moreover, low MBL and low M-ficolin levels have been found to be protective factor while low MASP2 (MBL associated serine protease-2) was observed to be susceptible factor against *M.leprae* infection [49]. Conflicting observations from earlier investigations resulted in unclear understanding of lectins in intra and extracellular pathogenic infections. Till date, no simultaneous investigations have been conducted to elucidate the possible role of lectins in intra and extracellular infections. We focused our investigations on parasitic diseases as the knowledge of lectins in parasitic diseases was limited and it is ambiguous whether lectins are of factual importance to human host during parasitic infections [50].

3. Parasitic Diseases

A diverse range of parasites can infect humans, from unicellular protozoan to multicellular metazoan organisms, such as worms. The major human parasitic diseases

are malaria, schistosomiasis, African trypansomiasis, leishmaniasis, Chagas' disease, lymphatic filariasis and onchocerciasis [51]. Parasitic diseases are a significant threat to global health and causes more than a million deaths every year [52]. It is also associated with significant morbidity in terms of DALYs (Daily Adjusted Life Years) and has a large economic impact in tropical and subtropical regions of the world including Africa, South America and Asia [53]. Parasite diseases are directly associated with poverty and affects largely the poor people with low income [54].

3.1 Complement Lectin pathway activation and Parasite Immune Evasion

Parasites, upon successfully crossing an anatomical barrier of the host, immediately face the complement attack by lectins due to its rapid immune response [10]. The activated lectin pathway directs many immune effector functions, that ultimately result in destruction of invading parasites via effector molecules, such as the anaphylatoxins (C3a and C5a), opsonins (C3b/iC3b) and the lytic terminal complement complex (TCC) [55]. Many parasites including *Leishmania spp.*, *Plasmodium spp.*, *Trypanosoma cruzi, Schistosoma mansoni* were observed to bind to the lectin pathway initiating molecules such as MBL and ficolins and activates the lectin pathway [56-59]. However, parasites have evolved numerous immune evasion strategies to escape from complement attack. For example, *T.cruzi* expresses specific complement receptors (complement C2 receptor inhibiting trispanning-CRIT, calreticulin, Gp58/68) that inhibit the complement cascade [10]. On the other hand, in *Leishmania spp.*, enhance the complement activity by the conversion of active C3b into inactive C3b (iC3b) through glycoprotein 63 (Gp63), a C3b acceptor that induce parasite phagocytosis by macrophages. In addition, amastigotes enhance their cellular uptake by targeting complement receptor 3 (CR3) [60]. *Schistosoma mansoni* inhibit complement attack by expressing its constitutive components such as schistosoma complement inhibitory protein-SCIP, C3 receptor, ectoproteases and CRIT. *Plasmodium falciparum* infection needs balance between the activation and regulation of the complement system to determine the severity and outcome of malaria. Recent findings demonstrated that C5a modulated in placental and cerebral malaria [61, 62].

4. Extra cellular parasitic infection-Schistosomiasis

Schistosomiasis (Bilharziasis), a chronic parasitic disease caused by *Schistosoma* trematode worms. The disease is largely categorized into two forms viz. intestinal schistosomiasis and urogenital schistosomiasis based on the site of infection in human host. *S. mansoni, S. japonicum, S. mekongi* and *S. intercalatum* are the four species that causes intestinal schistosomiasis. Urogenital schistosomiasis is caused by *S. haematobium* [63]. Schistosomiasis is a poverty associated neglected tropical disease and occurs largely in parts of Africa, South America, and Asia [64]. Globally there are over 207 million people infected with the disease and an estimated of 779 million people are at risk of schistosomiasis [65]. Approximately 93% of the world's schistosomiasis cases occur solely in sub-Saharan Africa [66] and is considered as the second most socio-economically devastating tropical parasitic disease next to malaria [67].

4.1 Urogenital schistosomiasis

Urogenital schistosomiasis is endemic in many parts of sub-Saharan Africa (SSA). Nearly two-thirds of the cases of schistosomiasis in SSA result from urinary tract infections caused by the multicellular trematode parasite *S. haematobium* [68]. Adult worms reside in the veins of the urinary bladder plexus of the pelvic region where they lay eggs daily. These eggs subsequently penetrate the vessels and move towards the genital tract. The encountered eggs are sequestered by the human immune system and cause granuloma formations in the urinary bladder, lower ureters, cervix, vagina, prostate gland, and seminal vesicles [69]. The clinical manifestations of schistosomiasis include anemia, hematuria, hydronephrosis, upper urinary tract lesions and granulomatous inflammation in the genital tract [70, 71]. Chronic inflammation pathology in the affected organs leads to pelvic pain, post-coital bleeding and ulcerations of genitalia in women where as ejaculatory pain, hematospermia and leukocytospermia in men.

Epidemiologically, *S. haematobium* and HIV prevalence geographically overlap in Africa due to the damaging effects of urogenital schistosomiasis in the genital tracts

[72]. The epithelial ulcers caused by *S. haematobium* infection have been suggested to ease viral entry and observed to be a potential risk factor for acquisition of HIV [73]. However, strong gender bias was observed where women are more likely to get HIV infection due to urogenital schistosomiasis (Female genital schistosomiasis-FGS) than men. The disease is also linked to horizontal transmission of HIV as the sandy patches of FGS increases the susceptibility and Th2 directed CD4+ cells provide the shelter for HIV viral entry and replication. Therefore it may represent one of Africa's most important cofactors for the AIDS epidemic [74]. Bladder cancer is another severe complication of urogenital schistosomiasis. Individuals with urogenital schistosomiasis may develop bladder cancer much earlier than uninfected people. The severity of the disease is associated with intensity of infection, worm burden and tissue egg burden, and the duration of infection [75]. *S. haematobium* is classified as a Group 1 carcinogen by the World Health Organization (WHO) because it deposits eggs in the urinary bladder of infected people that leads to bladder cancer [76, 77]. The standard diagnostic test for active urogenital schistosomiasis is microscopical examination of viable eggs in urine and praziquantel is the standard drug of treatment. The limited supply of praziquantel, treatment gaps and rapid reinfections increases the disease burden [78].

4.2 Life cycle of *Schistosoma haematobium*

The life cycle of *S. haematobium* (Figure 2) begins once humans contact freshwater-harbouring cercariae. The infectious cercariae penetrate the human skin by their glandular secretions to reach the blood vessels and migrate to heart, lung, and liver and finally ends in the venous plexus of the bladder. The incubation period takes about 5-7 weeks; during the period schistosomula larvae mature into female and male adult worms and live as a permanently embraced couple. Adult female worms produce 300 - 3000 of eggs per day and shed them into the environment via urine. On contact with fresh water, the egg releases the miracidium, which searches for their intermediate snail host (*Biomphalaria and Bulinus*).

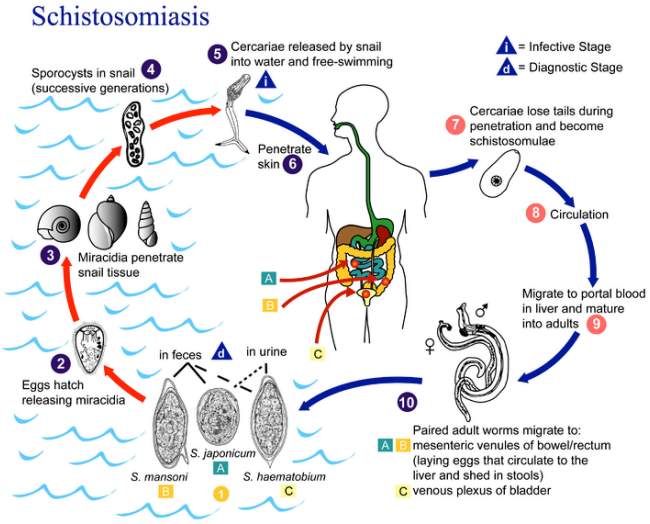

Figure 2: The life cycle of *Schistosoma haematobium* (source: www.cdc.gov). Freshwater snails serve as intermediate host. Cercariae (infective form) released by snail penetrate human skin and undergo structural changes and migrate to venous plexus of the bladder and further pair and produce eggs.

Once inside the snail, the parasites undergo asexual replication (sporocysts) and produce thousands of infectious cercariae that are ready to infect a new host [78]. Several factors including snail populations, cercarial density and patterns of human contact were observed to result in a focal distribution of the infection within countries, regions, and villages [70]. Children in early adolescence are at higher risk as they constantly come into contact with cercariae-infected water during their daily activities [79]. Additionally, certain occupational groups (fisherman, irrigation farmers) and women who need to access fresh water for their daily activities also possess greater risk of infection [78].

4.3 Lectins and schistosomiasis

Schistosomes surface is covered by fucosylated tegument that consist of various glycoconjugates and carbohydrate-binding proteins in all life stages [80]. The tegument of schistosomes has been characterized by lectin-binding studies [81, 82]. MBL recognized the *S. mansoni* adult and cercariae worms and activated the complement

system *in vitro* in C1q deficient serum [59]. In addition, ficolin-2 serum levels and *FCN2* gene polymorphisms were observed to confer relative protection against urogenital schistosomiasis [83]. These observations signify the role of the lectins in schistosomiasis.

5. Intra cellular parasitic infection-Leishmaniasis

Leishmaniasis, a vector-borne neglected tropical disease, is caused by caused by obligate intra-macrophage protozoa parasites from over 20 *Leishmania spp.* [84]. The disease is transmitted to human hosts by the bite of a blood sucking female phlebotomine sand fly. The disease is characterized by four distinct clinical manifestations viz. Cutaneous Leishmaniasis, Muco-cutaneous Leishmaniasis, Visceral Leishmaniasis (kala-azar) and Post Kala-azar Dermal Leishmaniasis (PKDL) [85]. Leishmaniasis has strong links with poverty and is associated with malnutrition, lack of resources to pay diagnosis and treatment, lack of bed nets, more prone to vector bites [86]. The disease is endemic in areas of the tropics, subtropics, southern Europe and temperate regions of some 88 countries [84]. According to World Health Organization (WHO) report, an estimated of 1.3 million new cases and 20 000 to 30 000 deaths occur annually due to leishmaniasis. An estimated of 310 million people are at risk of Leishmaniasis [87].

5.1 Visceral Leishmaniasis

Visceral Leishmaniasis (VL) is the most severe form of leishmaniasis and 100% fatal if left untreated. As the name denotes, the disease affects visceral organs including spleen, liver and lymph nodes [88]. VL is caused by *Leishmania donovani* (family: *Trypanosomatidae*, Order: *Kinetoplastida*) in East Africa and in the Indian subcontinent, whereas *Leishmania infantum* is the causative agent in North Africa, Latin America and Mediterranean regions of Europe. However, *L. infantum* infects mostly children and immunosuppressed individuals, whereas *L. donovani* infects all age groups [85, 89]. The common symptoms for VL are splenomegaly, hepatomegaly, anemia and weight loss. However, splenomegaly is often a typical symptom for VL [90].

VL ranks second in mortality next to malaria among tropical diseases with an estimation of 50,000 deaths per annum [91] and accounts for more than 2 million disability adjusted life years (DALYs) lost [92]. PKDL is a chronic, dermal sequel of VL that occurs in some patients who are cured from VL and limited mainly to Indian sub-continent and Africa. Though low mortality rates are reported, PKDL plays a major role in inter-epidemic transmission of VL due to the rich parasite density in dermal lesions [93]. Although VL is prevalent worldwide, the majority (≥90%) of cases occur in the following six countries viz. Bangladesh, India, Nepal, Sudan, Ethiopia and Brazil [85]. VL is considered as a major health problem in Indian sub continent (India, Nepal and Bangladesh) with an estimation of 150 million people living at risk and shares 67% of the global disease burden.

In India, VL is endemic in Central-East India (Bihar, West Bengal and Eastern part of Uttar Pradesh) and accounts for more than 90% of all Indian VL. The Bihar state of India shares almost 50% of the global VL burden and is observed to be a “hot spot” of visceral leishmaniasis [85, 94-96]. Microscopical visualization of amastigotes in splenic aspirates is the standard diagnostic method for VL. However, rK39 rapid diagnostic test (RDT) strip can substitute invasive splenic aspirates in Indian VL. Antimonial treatments are the drug of choice to cure VL except in Bihar where antimony treatment was ineffective and replaced with Amphotericin B [97]. Changes in vector control practices and therapeutic modalities were responsible for increased incidence of VL and related mortality in India [98].

5.2 Life cycle of *Leishmania donovani*

The life cycle of *L. donovani* (Figure 3) exists in two different developmental stages: the extracellular promastigotes in the invertebrate host (sand fly), and the intracellular amastigotes in the vertebrate (mammals) host. Female phlebotomine sandflies transmit the disease during their blood meal, by inoculating metacyclic promastigotes into the skin of the human host.

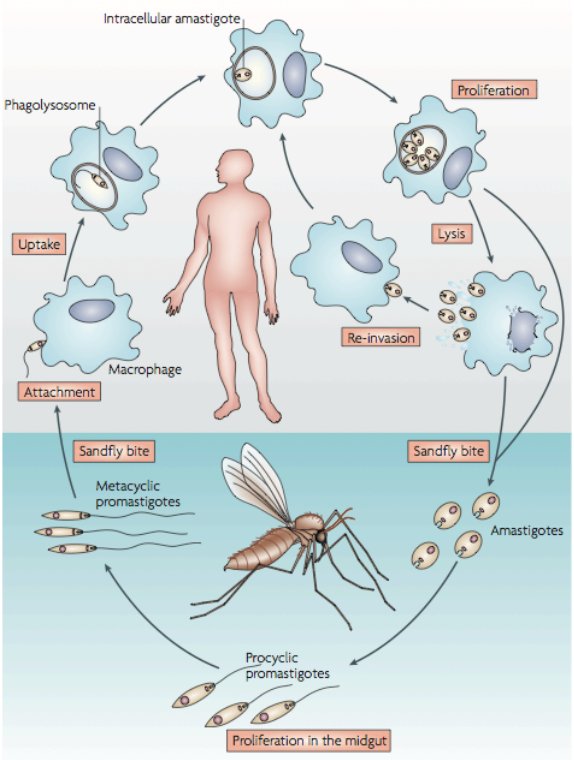

Figure 3: The life cycle of *Leishmania donovani* adapted from Ref. [85]. The female phlebotomine sandflies transmits the promastigotes into human host and parasites were phagocytosed by macrophages. Inside the cells they transform into amastigotes and replicates.

The parasites are phagocytosed by macrophages, monocytes, and langerhans cells where they differentiate into amastigotes [85, 99]. Upon their internalization by macrophages and other cells, promastigotes inhibit phagolysosome biogenesis and multiply inside the cell rather than getting lysed [100]. In the host, infected macrophages migrate from skin to visceral organs like the spleen, the liver and give rise to pathologies associated with VL. During the blood meal sandflies pick up amastigotes and releases them into their midgut where parasites differentiate into procyclic promastigotes and followed by metacyclic promastigotes. Finally differentiated metacyclic forms are ready for the transmission to the next vertebrate host by sand-fly bite [99].

5.3 Lectins and Visceral Leishmaniasis

Leishmania spp., is enveloped with mannose-containing lipophosphoglycan (LPG) and mannose glycoinositol-phospholipids [58]. These glycoconjugates are recognized by the lectins such as the mannose-binding lectin. Earlier study reported that MBL binds to *L. braziliensis* mediated by a specific carbohydrate on the surface of parasites [101]. MBL was observed to enhance the susceptibility to VL [102-104]. However, these studies were focused on the South America and Africa with infections caused by *L. chagasi* and *L. infantum* with limited sample size. No investigations were reported on the role of lectins (MBL and ficolins) in VL caused by *L. donovani* that responsible for more VL cases.

6. Interleukin-6 regulates acute phase response of lectins in infections

IL-6 is an important cytokine that regulates a various immunological functions during distinct infections [105]. IL-6 stimulates the expression of acute phase proteins in hepatocytes by binding to IL-6-responsive elements in the promoter region of the respective genes [106]. MBL is such an acute-phase plasma protein (APP), and is regulated tightly by IL-6 [107]. MBL is a serum lectin that initiates the complement activation. Moreover, MBL deficiency is associated with a variety of diseases [14, 108-110]. The *MBL2* promoter region consists of type 1 and type 2 IL-6-responsive sequences [24]. Therefore, it is anticipated that IL-6 regulates the MBL in infections and inflammation. In a patent infection, schistosomes induce strong Th2 immune response through increased production of IL-6 that down-regulate Th1 immune response [111]. In addition, the cercariae are recognized by host macrophages that induce the secretion of IL-6 [112]. IL-6 was observed to be protective factor against *Schistosoma*-induced pulmonary hypertension *in vivo* [113].

Leishmania donovani is the causative agent of VL in India with the predominance of Th2 immune response in active infections whereas protection is associated with Th1 immune response [114]. IL-6 levels were consistently elevated in active VL cases and appear to play a central role in pathogenesis of VL [108, 115, 116]. IL-6 was observed

to favor parasite survival in human macrophages as it down modulates the cytokine-enhanced anti-leishmanial activity [117]. The functional role of IL-6 and MBL has been addressed in schistosomiasis and VL independently. However, the role of IL-6 in regulation of MBL expression in these diseases was not investigated. Therefore, we studied the role of IL-6 and its relation with MBL regulation in urinary schistosomiasis and visceral leishmaniasis. As ficolins and Collectin-Kidney-1 (CL-K1) share similarities in both their structure and function with MBL [28], we also studied the impact of IL-6 in the expression of these proteins in the investigated diseases.

OBJECTIVES

The role of the lectins in parasite infections is controversial and is context dependent. The investigated studies aimed to analyze the functional role of lectins in intra and extracellular parasitic infections. The objectives are,

1. In the first part of the thesis, we aim to investigate the possible genetic and phenotypic associations of the lectins mannose-binding lectin (MBL), collectin-kidney 1 (CL-K1), and the downstream cleaving enzyme MBL associated serine protease 2 (MASP-2) with urinary schistosomiasis in a Nigerian study group.
2. In the second part of the thesis, we aim to study the possible genetic and phenotypic associations of the lectins MBL, ficolin-2 with visceral leishmaniasis in an Indian study group.
3. In addition, we aim to investigate the induction of acute phase responses by IL-6 in lectins expression during urinary schistosomiasis and visceral leishmaniasis.

STUDY GROUP

Urinary schistosomiasis: To represent extracellular parasitic infection we utilized a Nigerian study group consisting of *Schistosoma haematobium* infected patients (schistosoma egg positive in urine, SEP; n=168) and two control groups of individuals who previously exposed (schistosoma ELISA positive, SELP; n=123) and never exposed (schistosoma ELISA negative, SELN; n=69).

Visceral leishmaniasis: To represent intracellular parasitic infection we used an Indian study group consisting of *Leishmania donovani* infected individuals (n=218) and healthy subjects (n=225).

METHODS

All the functional genetic variants in investigated lectin encoding genes were genotyped by direct sequencing and corresponding serum levels were measured by ELISA.

RESULTS

Manuscript 1: Mannose-binding lectin and susceptibility to schistosomiasis.

Antony JS et al. Mannose-binding lectin and susceptibility to schistosomiasis. *J Infect Dis* 2013, 207(11):1675-1683.

The serum MBL levels were higher in control groups (SELP+SELN) when compared to schistosomiasis positive (SEP) cases (*P*<0.0001). The serum MBL levels of the reconstructed *MBL2*HYPA* haplotype in SEP cases was observed to be lower when compared to SELP control group (*P*<0.0001) as well as with pooled controls (SELP+SELN) (*P*<0.0001). All the analyzed functional *MBL2* SNPs in investigated groups were in Hardy-Weinberg equilibrium. Linkage disequilibrium pattern revealed that the 6-bp (-338 to -332) deletion in the promoter region was in linkage disequilibrium with the promoter variant +4C/T (P/Q) in all studied sub groups. The homozygous variant *LL* (-550CC) genotype was observed more frequent in SEP case group in comparison to controls (SEP vs. SELP+SELN: OR=2.1, 95%CI=1.06-4.59, *P*=0.03) inferring an increased risk to *S. haematobium* infection. The heterozygous genotype *HL* (-550GC) was observed less frequent in SEP case group compared to both control groups (SEP vs. SELP+SELN: OR =0.44, 95%CI=0.20-0.91, *P*=0.03) contributing towards a protective factor to *S. haematobium* infection. The minor allele -550*H* was observed more in control group (SEP vs. SELP+SELN: OR=0.5, 95%CI=0.24-0.98, *P*=0.03) suggesting a shielding factor against urinary schistosomiasis.

In exon1, heterozygous genotype *PQ* (+4CT*)* was observed more frequent in SEP case group in comparison to SELN control (SEP vs. SELN: OR=2.27, 95%CI=1.2-4.31, *P*=0.02). The homozygous genotypes *PP (*+4CC*)* were observed less in the SEP cases group compared to SELN (OR=0.4, 95%CI=0.2-0.9, *P*=0.02). The heterozygous 6-bp deletion was observed to be more in SEP cases when compared to SELN (OR=2.15, 95%CI=1.1-4.09, *P*=0.01). The reconstructed *MBL2*HYPA* haplotypes was associated with protection (SEP vs. SEP vs. SELP+SELN: OR=0.5, 95%CI=0.25-98, *P*=0.03).

Manuscript 2: Lectin complement protein Collectin 11 (CL-K1) and susceptibility to urinary schistosomiasis.

Antony JS et al. Lectin complement protein Collectin 11 (CL-K1) and susceptibility to urinary schistosomiasis. *PLoS Negl Trop Dis* 2015, 9(3):e0003647.

The mean circulating CL-K1 serum levels among healthy Nigerian individuals were 246±155 ng/mL. The circulating CL-K1 serum levels were differentially distributed between the investigated groups (SEP=175 ng/mL vs. SELN 246ng/ml, *P*=0.0004; SEP 175 ng/mL vs. SELP+SELN 198 ng/mL, *P*=0.039). The *COLEC11* non-synonymous variant (*rs7567833-AA, p.216H*) in exon8 was found to be higher in SELN healthy controls (OR=0.2, 95%CI=0.08-0.9,P^{corr}=0.01) conferring a decreased risk for urinary schistosomiasis. Similar contributions were also observed in different genetic models [(Allelic model: OR=0.44, 95%CI=0.22-0.72, P^{corr}=0.0004); (Dominant model: OR=0.42, 95%CI=0.22-0.79, P^{corr}=0.0048); (Recessive model: OR=0.2, 95%CI=0.08-0.9, P^{corr}=0.01)]. The other variants in the promoter region were not significantly associated. The distribution of reconstructed *COLEC11*TCCG* haplotype (with all major alleles) was observed more frequent in SEP cases than in SELN healthy controls (OR=1.76, 95%CI=1.15-2.70, P^{corr}=0.007).

The *COLEC11*TCCA* haplotype with p.R216H substitution was observed more frequently in SELN controls [SEP vs. SELN: OR=0.38, 95%CI=0.23-0.63, P^{corr}=0.0001; SEP cases vs. SELP+SELN controls: OR=0.66, 95%CI=0.43-0.99, P^{corr}=0.04)]. The minor allele of exon 8 variant *rs7567833A* (*p.216H*) was significantly associated with increased CL-K1 serum levels. A gene dose dependent effect on the distribution of serum CL-K1 levels was observed. The *COLEC11*TCCA* haplotype contributed to low serum CL-K1 levels in the SEP cases (*P*<0.0001). Furthermore, individuals with *COLEC11*TCCG* haplotype was observed to have lower CL-K1 serum levels when compared to other haplotypes in investigated control groups but not in SEP cases.

Manuscript 3: Correlation of Interleukin-6 levels and lectins during *Schistosoma haematobium* infection.

Antony JS et al. Correlation of Interleukin-6 levels and lectins during *Schistosoma haematobium* infection. *Cytokine* 2015. doi:10.1016/j.cyto.2015.04.019

IL-6 serum levels among combined (SELP+SELN) controls varied from 0.2 to 18.9 pg/ml with a median of 1.8 pg/mL. IL-6 serum levels were elevated in *S. haematobium* egg-positive (SEP) cases (mean 8.7 pg/ml) when compared to the both control subgroups separated or merged. (SELP: mean 4.9 pg/ml, SELN: mean 5.9 pg/ml, SELP+SELN: mean 5.3 pg/ml: $P < 0.0001$). We further correlated IL-6 serum levels with those of the previously reported lectins (MBL, ficolin-2, CL-K1) in both SEP cases and control subgroups. IL-6 serum levels were positively correlated with MBL (Spearman's rho coefficient: $\rho=0.189$, $P=0.01$), ficolin-2 ($\rho=0.192$, $P=0.01$) and CL-K1 ($\rho=0.292$, $P=0.0002$) in cases. Notably, IL-6 was inversely correlated with MBL ($\rho=-0.239$, $P=0.001$) and ficolin-2 ($\rho=-0.239$, $P=0.001$) in the pooled control subgroups. CL-K1 did not show any significant correlation with IL-6 in controls. A similar correlation was observed when the control subgroups were analyzed separately.

To validate the association of IL-6 with lectins production, similar investigation was performed in visceral leishmaniasis, an intracellular parasitic disease. IL-6 levels were measured in VL cases (n=58) and controls (n=30) with the similar experimental settings and correlated with lectins MBL and ficolin-2 levels. IL-6 levels were elevated in VL cases (mean 7.07 pg/mL) compared to controls (mean 3.1 pg/mL) ($P = 0.003$). In VL cases, the MBL levels were positively correlated with IL-6 (Spearman's rho coefficient: $\rho=0.3$, $P=0.01$) while ficolin-2 did not show any significant correlation with IL-6. No correlation was observed between IL-6 and lectins in control subjects.

Manuscript 4: Association of Ficolin-2 serum levels and *FCN2* genetic variants with Indian visceral leishmaniasis

Mishra A and Antony JS et al. Association of Ficolin-2 serum levels and *FCN2* genetic variants with Indian visceral leishmaniasis. *PLoS One* 2015, 10(5):e0125940.

Ficolin-2 serum levels were elevated in VL cases (mean 2.77 μg/ml) when compared to healthy controls (mean 1.94μg/ml) (adjusted *P*<0.0001; corrected for age, sex and ethnicity). All investigated variants (-986G/A, -602 A>G, -4 A>G and +6359 C>T) in VL patients and controls were in Hardy-Weinberg equilibrium (P<0.05) except for one studied +6424 G>T (rs7851696) variant in VL cases and excluded for further analyses. Both in genotype and allele distributed with significant differences between VL cases and controls for the non-synonymous variant +6359C>T (p.T236M). The homozygous genotype +6359-TT occurred more frequently among VL cases compared to controls after adjusting for age, sex and ethnicity (OR=2.2, 95%CI=1.23-7.25, *P*=0.008), indicating that this variant was associated with susceptibility with VL. The similar effect was observed with different genetic models [Allelic: OR=1.4, 95%CI=1.02-1.94, *P*=0.03; Recessive: OR=2.2, 95%CI=1.23-7.25, *P*=0.008]. However, +6359 C>T was observed to be in strong LD with -986 G/A, and -4 A>G but not with -602 A>G. Other investigated *FCN2* variants were not significantly associated with visceral leishmaniasis. The distribution of reconstructed haplotype *FCN2*AAAC* was found more frequently in healthy controls compared to VL cases (OR=0.59, 95%CI=0.37-0.94, *P*=0.023).

Manuscript 5: Low MBL-associated serine protease 2 (MASP-2) levels correlate with urogenital schistosomiasis in Nigerian children.

Ojurongbe O and Antony JS et al. Low MBL-associated serine protease 2 (MASP-2) levels correlate with urogenital schistosomiasis in Nigerian children. *Trop Med Int Health* 2015. 20(10):1311-1319.

All investigated functional *MASP-2* variants in each subgroup were in Hardy-Weinberg equilibrium, except for the variant *rs2273346* (p.V377A) in SEP cases, which was excluded from further analyses. No significant contribution of genotypes or alleles was observed. The exon 3 variant rs72550870 (p.D120G*)* was monomorphic in the population under study. A total of twelve haplotypes were observed at a frequency >2% in our study population. However, the frequencies of the observed secretor haplotypes did not differ between subgroups (SEP cases vs. SELP, SELN controls; SEP cases vs. SELP+SELN controls).

The MASP-2 serum levels varied from 1.8 to 1073 ng/mL with a median of 106.9 ng/mL. The serum levels were not differentially distributed among our study subgroups (P>0.05). The multivariate analysis, however, revealed a confounding effect of age to MASP-2 serum levels (P=0.0001). We, therefore, grouped our study participants according to age and to the rationale that younger children might be rather at risk, as they have frequent contact with water infested by the infectious form of schistosomae, the cercariae. Based on an average puberty age of 12 years in the population studied, the subjects were separated into the groups of ≤12 years and >12 years of age. We observed that MASP-2 serum levels were lower in children ≤12 years with a patent infection (SEP) compared to those with previous infections (SELP) (P=0.0074). MASP-2 serum levels were higher in infected (SEP) older children and adults (>12 years) compared to those who were exposed previously (SELP) (P=0.032). The correlation analyses were performed for the MBL and ficolin-2 serum levels described previously for the same study group with the circulating MASP-2 serum levels assessed here. We noticed that only MBL serum levels were positively correlated with MASP-2 serum levels (r=0.39, P=0.01).

Manuscript 6: Mannose-Binding Lectin (MBL) as a susceptible host factor influencing Indian visceral leishmaniasis.

Mishra A and Antony JS et al. Mannose-binding lectin as a susceptible factor influencing Indian visceral leishmaniasis. *Parasitol Int* 2015, 64(6):591-596.

Mean circulating MBL serum levels among Indian healthy individuals were 109.6±143 ng/ml. The MBL2 serum levels in the study group varied from 0.44 to 911 ng/ml with a median of 122.5 ng/mL. Circulating MBL serum levels were heterogeneously distributed between visceral leishmaniasis (VL) cases and healthy controls (median VL cases=155.1 ng/mL vs Controls =78.64 ng/mL, *P*=0.007 after adjusted for age, sex and ethnicity). Both genotype and allele frequencies for all of the studied *MBL2* variants in each subgroup were in Hardy-Weinberg equilibrium except for variant rs10556764 (-338 to -332 6bp deletion) in VL cases and subsequently excluded for further analysis. The promoter variants -78C/T and +4P/Q are in strong LD with each other in both VL cases and controls. The frequency of *MBL*2 minor alleles in the promoter region *-78T* was observed higher in healthy controls compared to VL cases after adjusted for age, sex and ethnicity (OR=0.7, 95%CI=0.5-0.96, adjusted *P*=0.026). In addition, the distribution of another promoter variant *+4Q* in the transcription start site was observed higher in the healthy controls compared to VL cases after adjusted for age, sex and ethnicity (OR=0.66, 95%CI=0.48-0.9, adjusted *P*=0.012). Both observations were consistently significant with the dominant genetic model.

The *MBL2*LYQA* haplotype was observed more frequently in healthy controls than in cases (OR=0.69, 95%CI=0.5-0.97, adjusted *P*=0.034). Serum MBL levels were associated with functional *MBL2* genotypes. The minor alleles *rs11003123T, rs7095891T (+4Q)* and *rs4647964A* contribute to significantly increased MBL serum levels, whereas the major alleles *rs11003123C, rs7095891C (+4P)* and *rs4647964G* contribute to a significantly decreased MBL serum levels in healthy controls. Serum MBL levels were in accordance with their respective diplotypes in VL patients (P<0.0001).

DISCUSSION

Human resistance to pathogenic infections is mediated by humoral and cellular effector systems of innate immunity. The complement system is an essential component of the innate immunity and leads to the generation of opsonic, chemotactic, and lytic functions against invading pathogens. The complement proteins deficiency leads to disease susceptibility and to recurrent infections [118]. The success in infecting the host by pathogens is dependent on their capacity to resist the complement attack, either by inhibiting the complement cascade or by escaping its activation [119]. Complement system consists of more than 40 circulating serum proteins including group of pattern recognition molecules, cleaving proteases enzymes, complement components, receptors and regulators [120]. These components interact in a complex cascade of events including enzymatic cleavage of molecules and dramatic conformational changes generating biologically active molecules. Upon recognition of pathogens, the complement system is activated via one or more of the following three pathways namely classical, lectin and alternative pathway. Though the complement lectin pathway uncovered later, it is shown to be crucial in recognition and clearance of parasites compared to classical and alternative complement pathways [10].

Though the lectin deficiency associated with increased susceptibility to several infections [41-43], it is also reported to be a protective factor in intracellular infections [44-46]. However, conflicting observations were reported among functionally similar lectins in intracellular infections and warranted further investigations [48]. The impact of lectin deficiency on parasitic infections is limited. Therefore, in this thesis, we studied the interactions host lectins in intra and extra cellular parasitic infections.

1. Lectins in extracellular *S. haematobium* infection

Schistosomiasis is a multifactorial disease which depends on environmental, behavioral, parasitic, vector and host factors [121]. Furthermore, individuals from certain families possess the highest infection levels and are more susceptible to severe reinfections suggesting that host genetic factors may play a possible role in resistance to

Schistosoma infections [122, 123]. Human genome scans in 11 Brazilian families have identified an SM1 locus responsible for controlling *S. mansoni* infection intensity on chromosome 5q31–q33 [124] and subsequently were reconfirmed in the Senegalese population [125]. Many immune response genes, including several Th2 specific cytokines (interleukin (IL)- 3, IL-4, IL-5, IL-9, IL-13), interferon regulatory factor 1, colony-stimulating factor 1 (CSF-1), CSF-2 and the IL-12/IL-23 p40 subunit were mapped in this region and observed to be a major genetic susceptible loci for schistosomiasis [126]. In light of these earlier observations, it is evident that the host genetic make up is a crucial factor to resist schistosoma infections.

Earlier observations documented that lectins recognize the *Schistosoma* tegument surface and initiate complement mediated innate immune response [59]. Therefore, in the current study we evaluated lectins serum levels and respective functional variants against urinary schistosomiasis in a Nigerian study group. A previous study on ficolin-2 using the same study group revealed that *FCN2* gene polymorphisms and ficolin-2 levels influence the *S.haematobium* infection [83]. In the present study we extended our investigations to other important lectins such as Mannose-binding Lectin (MBL), Collectin-kidney 1 (CL-K1) and MBL Associated Serine Protease 2 (MASP-2) in order to study their functional role in urinary schistosomiasis.

In our study, the influence of MBL serum levels and *MBL2* functional polymorphisms to urinary schistosomiasis susceptibility was assessed. Data revealed that MBL serum levels were higher in the control groups compared to the infected individuals indicating a protective role of MBL against *S. haematobium* infection. Our results are in line with previous observations where MBL was reviewed as a key component of the innate immune system by protecting persons against a broad range of infectious diseases caused by bacteria, fungi, viruses, and parasites [22]. Our genotype results showed that three variants in *MBL2* gene (-550G/C, +4C/T and 6bp deletion at -338 to-332) play a role in influencing susceptibility to schistosomiasis. The major allele -*550L* contributed towards increased susceptibility whereas -*550H* minor allele in the studied population decreased the risk of *Schistosoma* infection. The distributions of heterozygous genotypes (+4CT and wt/6bp-del) were higher in the cases in comparison

to controls revealing a higher risk of getting schistosomiasis. *MBL2* haplotype analysis showed that the *MBL2*HYPA* haplotype occurred less frequently in the cases group than in controls elucidating the protective effect of this high MBL secreting haplotype towards schistosomiasis. Similar results on the influence of *MBL2*HYPA* haplotypes have been demonstrated on pediatric patients with common infectious diseases and reduced risk of infection following allogeneic hematopoietic stem cell transplantation [127, 128].

We extended our investigations to evaluate the association of Collectin-kidney 1 (CL-K1) with urinary schistosomiasis as Schistosome teguments are fucosylated and CL-K1 has higher binding affinity to fucose [129, 130]. This is a primary study that examined the role of CL-K1 in an infectious disease context. The current study demonstrated that CL-K1 serum level was higher in the control group compared with infected individuals, showing that high levels of CL-K1 contributes protective effect to *S. haematobium* infection. The result was in line with previous observation on MBL and ficolin-2 using the same study group. Genotype association analysis revealed that the non-synonymous substitution in the exon8 (p.R216H) contributes to reduced schistosomiasis susceptibility. In specific, the minor allelic variant (p.216H) reduces the infection risk and the result was consistent with different genetic models employed. In addition, allele *p.216H* was associated with higher CL-K1 serum levels in healthy individuals. Of interest, rs7567833G/A (p.R216H) variant was reported to be under selective pressure and is differentially distributed in world populations [131, 132]. Other observed polymorphisms in the promoter and coding regions did not contributed to the disease susceptibility. *COLEC11* haplotypes revealed that *COLEC11*TCCA* haplotype confers a shielding effect to *S. haematobium* infection. In light of these findings, it is evident that higher CL-K1 serum levels and the *p.216H* allele confer a protective effect to the host against urinary schistosomiasis.

Further, we investigated MBL-associated serine protease 2 (MASP-2) utilizing the same study group, as it is a direct downstream protein of MBL and ficolins in lectin complement pathway [133]. We hypothesized that the MASP-2 complement protein is as equally important as MBL, CL-K1 and ficolin-2 to resist against *S. haematobium*

infections and thus MASP-2 serum levels and *MASP2* variants were evaluated. No significant contributions of investigated *MASP2* variants to schistosomiasis susceptibility were observed. The MASP-2 serum levels were not differentially distributed in investigated groups, but age was observed to be a confounding factor influencing MASP-2 serum levels, hence the data was segregated based on the mean puberty age (12 years) in the study population [134]. An age related pattern was observed in the distribution of MASP-2 levels in patent *S. haematobium* infected individuals. Infected children (<12 years) had lower serum MASP-2 levels compared to unaffected children. On the other hand, infected older children and adults had higher serum MASP-2 levels compared to control subjects with similar age. Similar age-related patterns in urinary schistosomiasis have also been identified in studies on other immune components such as myeloid dendritic cells (mDCs) and distinct cytokines [135-137]. The experimental infection of *S. mansoni* in mice either before or after puberty showed that pre-puberty infected mice had a higher number of adult worms and higher mortality rates as compared to post-puberty infected mice [138]. A clear association of age and intensity of infection is predicted in the field. In endemic areas, children below puberty age are more likely to get an *S. haematobium* infection than adults due to the lack of acquired protective immunity [139]. Based on our observations on MASP-2 and its relation with puberty age, we agree with the hypothesis of 'Puberty and Resistance to Schistosomiasis' proposed by Fulford *et al*., as it suggests that host age needs to be considered to achieve elimination of schistosomiasis [140].

Overall, our findings support the notion that lectins MBL, CL-K1 and their functional variants may be protective host factors in urinary schistosomiasis. We also observed that MASP-2 serum levels were associated with age and relative protection from urinary schistosomiasis in Nigerian children.

2. Lectins in intracellular *L. donovani* infection

Visceral Leishmaniasis (VL) is a complex disease phenotype determined by multiple factors such as the environment, the insect vector, and the parasite and host genetics. The contributing host genetics involves multiple immune genes towards disease

susceptibility [141]. Notably, 80–90% of human *L. donovani* infections are sub-clinical or asymptomatic, usually associated with host immunity. Additional to other factors, genetic risk factors determine why two people with the same exposure to infection differ in susceptibility [142]. Recent genome-wide association studies (GWAS) from Brazil and India with larger sample size have identified a strong association *HLA-DRB1–HLA-DQA1* locus in both populations irrespective of the geographical distance and different causative parasite species [143]. The role of host genetics in VL susceptibility reviewed by *Sakthianandeswaren et al.,* by exploring published literatures and concluded that many GWAS and candidate gene association studies demonstrated to the contribution of host genetics to resist against *L. donovani* infection [144].

Complement system has long been acknowledged as an important component of host defense to extra-cellular pathogens. However, their role in intra-cellular pathogens favors progression of the infection, as in *Leishmania*. As soon as the *Leishmania* promastigotes are injected by phlebotomine sandflies into the skin, the parasite has to face two of the most ancient yet effective immune mechanisms: (1) lysis by complement; and (2) destruction by phagocytes. *Leishmania* not only evades destruction by complement and phagocytes, but exploits the opsonic properties of complement to enhance its interaction with phagocytes, the host cells in which it replicates [145]. *Leishmania spp*., activates the lectin-complement pathway suggesting the active participation of lectins in VL [58, 101]. In current study, we investigated the possible contributions of *MBL2, FCN2* functional variants and circulating MBL, ficolin-2 levels in VL cases and controls from endemic region of Bihar, India.

The influence of MBL serum levels and *MBL2* functional polymorphisms to VL was assessed. Data revealed that MBL serum levels were higher in the VL cases compared to the controls indicating a susceptible role of MBL against *L. donovani* infection. Our observation is in line with earlier studies from Brazil that MBL level was increased in individuals with VL caused by *L. chagasi* compared to asymptomatic and uninfected individuals [44, 102]. Genotype analysis showed that two variants in promoter region of *MBL2* gene (-78C/T and +4C/T) were associated with decreased susceptibility to VL. The contribution of the variant -78C/T (rs11003123) to circulating

MBL level or associations in other diseases are limited. Reconstructed *MBL2* haplotypes revealed that the *MBL2*LYQA* haplotypes were distributed more frequently in healthy controls compared to VL patients, conferring a protective effect. The *MBL2*LYQA* haplotype is related to higher expression of serum MBL levels and associated with reduced risk in severe malaria patients from the Indian population [110].

In addition, we investigated ficolin-2 serum levels and *FCN2* variants to *L. donovani* infection in the same study group, as ficolin-2 is a functional analogue to MBL [146]. We observed that ficolin-2 serum levels were higher in VL cases compared to healthy controls conferring the susceptible role ficolin-2 to *L. donovani* infections similar to MBL. However, our observation was outlined with an earlier report where ficolin-2 levels were decreased in individuals with chronic Chagas disease caused by *Trypanosoma cruzi,* an intracellular parasite phlyogentically related to *L. donovani* [147]. Our results strongly suggest that high concentrations of ficolin-2 may enhance the uptake of *L. donovani* to host phagocytes/macrophages, the milieu preferred by these pathogens for their survival and replication. Genetic association analysis of *FCN2* variants with VL showed that the structural variant +6359 C>T (rs17549193, p.T236M) in fibrinogen like domain of *FCN2* gene was observed to increase the predisposition to visceral leishmaniasis. The computational prediction revealed that p.T236M substitution has major impact on the physiochemical property of the ficolin-2 and related to a significantly higher ficolin-2 serum level [148, 149]. Our *FCN2* haplotypes revealed that the *FCN2*AAAC* haplotype frequency was higher among controls than in VL cases, indicating that individuals with this haplotype had a diminished probability to develop VL.

Overall, we observed a high serum MBL and ficolin-2 levels in patients with VL inferring an active involvement of lectin pathway in visceral leishmaniasis. We also demonstrated that *MBL2, FCN2* variants are significantly associated with VL in the studied Indian study group.

3. IL-6 regulates lectins expression in parasitic infections

The pathogenesis of parasitic diseases is complex. Parasites, appear to initiate the acute phase plasma response during their migration and localized inflammation that is regulated by cytokines and hepatocyte-stimulating factors [150]. Cytokines are released in response to infection and are involved in many pathophysiological events. IL-6 is a pleiotropic cytokine and has the capacity to induce the expression of APP [107]. In pediatric patients with community acquired infections and bacteremia, the acute phase C-reactive protein was strongly correlated with IL-6 [151]. As MBL is a known APP [152], we hypothesized that IL-6 might exert synergistic effects with MBL and other lectins during *S. haematobium* and *L. donovani* infection [153]. Therefore, we studied the role of IL-6 in urogenital schistosomiasis and VL.

Our data indicate that IL-6 serum levels were higher among *S. haematobium* infected individuals compared to the *S. haematobium* sero-positive and sero-negative control subgroups. This observation is in line with earlier reports of natural and experimental *Schistosoma* infections [111, 154]. IL-6 serum levels have previously been shown to be also elevated in patients with *S. haematobium* induced bladder cancer and, correlated with advanced tumor grade and exhibited as a disease modifier [155]. Moreover, IL-6 was observed to interact with monocytes and platelets in killing of *S. mansoni* parasites [156]. Our findings emphasize the potential role of IL-6 in the pathogenesis of urinary schistosomiasis. Schistosomiasis is a strong inducer of a Th2 response with IL-6 expression.

We noticed a positive correlation of the lectins MBL, Ficolin-2 and CL-K1 with IL-6 in urinary schistosomiasis. Similar positive correlation was observed between IL-6 and the C-reactive protein (CRP) in HIV patients with the immune reconstitution inflammatory syndrome (IRIS) [157]. It is evident that MBL and other functional analogues may be modulated by IL-6 stimuli and essentially up-regulates the acute phase response proteins (APP) encoding genes [158]. However, in spite of the up-regulation by IL-6 of APP genes, we observed reduced MBL, Ficolin-2 and CL-K1 serum levels in *S. haematobium* infected individuals. We postulate that the schistosoma

parasite factors contribute to lower circulating serum levels of MBL, Ficolin-2 and CL-K1 by complement consumption. Related observation of MBL and IL-6 has been described in meningococcal infection [159]. In a cystic fibrosis patient who infused with recombinant MBL, MBL was observed to modulate the inflammatory reaction as IL-6 and CRP levels were inversely correlated with MBL serum levels [160]. Of interest, we noticed an inverse correlation of IL-6 with MBL and ficolin-2, but not with CL-K1 serum levels, both in seropositive and sero-negative controls as they did not exposed to inflammatory stimuli.

The observation of IL-6 and lectin expression in VL is similar to the schistosomiasis. IL-6 levels were elevated in VL cases compared to controls similar to earlier reports [108, 115, 116]. IL-6 seems to be a central cytokine in VL as it is associated with mortality, liver damage, and the regulation of other vital cytokines [108]. IL-6 was also observed to reduce the anti-leishmanial activity in human macrophages [117]. A positive correlation was observed between MBL and IL-6 in VL cases. The similar positive correlation of IL-6 and APP–CRP was observed in VL cases [108]. It is clear that likewise with CRP, MBL may be modulated by IL-6 stimuli in *L. donovani* infections. Moreover, the circulating serum levels of MBL were elevated in the same investigated samples. However, no association between ficolin-2 and IL-6 was observed in VL cases. No correlation of lectins with IL-6 was noticed in control subjects. We speculate that a larger sample size would corroborate the understanding of the contribution IL-6 in regulation of ficolin-2 expression in *L. donovani* infection as we measured the IL-6 in less number of samples. We have shown that IL-6 levels are elevated in infected individuals and that IL-6 levels regulate the production of lectins during the infection.

4. Are lectins friend or foe in parasitic infections?

Often, parasites exhibit very strong immune evasion strategies to escape from complement attack and to achieve the infections. Earlier studies investigated that, the complement system fails to kill the parasite, due to immune evasion or subversion (i.e., abusing the complement components to facilitate the infections) [50]. The

glycoconjugates presented by parasites are involved in the stimulation of non-specific innate immune response and recognized by host carbohydrate binding protein family of receptors including lectins in a calcium dependent manner. The lectin complement system is a primary part of the innate immunity that encounters parasites immediately upon invasion into the human host [10]. However, lectins deficiency is an essential host factor contributing to the infection susceptibility. The objective 1 and 2 presented in this thesis explored the role of the lectin pathway components in distinct parasitic infections.

4.1 MBL and parasitic diseases

The mannose-binding lectin (MBL) is a key recognition element involved in binding oligosaccharide structures exposed on parasites. MBL is a circulating serum protein constitutively present in the blood and act very immediate against the invaded parasites [10]. *Schistosoma,* an extra-cellular blood fluke trematode worms and are constantly exposed to MBL. In addition, MBL was shown to adhere to the surface glycoproteins of cercariae and adult worms [59]. Therefore, we investigated the effect of MBL in urinary schistosomiasis. The clinical relevance of our findings is that higher circulating MBL levels and high MBL expression genotype led to increased protection to *S. haematobium* infection in the investigated Nigerian study group. In contrast, a recent study reported that higher plasma MBL levels increase the susceptibility to *S. haematobium* infections in Zimbabwean study group [161]. The mechanisms behind these contrasting findings towards a role of MBL levels in urinary schistosomiasis are not clearly understood as both populations have African ethnicity and similar sample size. However, the authors justified that due to the numerous intracellular infections occurring in African population, might have exerted selection pressure to low MBL2 expression genotypes and thus reduced MBL plasma levels [162]. Moreover, experimental *Schistosoma* infection in MBL knockout (MBL-$A^{-/-}$) mice revealed that the course of infection was not altered, raising questions about biological relevance of MBL against schistosomiasis [163]. Nevertheless, we strongly rely on observations of MBL against schistosomiasis as we noticed a similar effect with other functional analogues such as ficolin-2 and CL-K1 in the same study group [83].

The extra-cellular living filarial worms *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* are the causative agents for lymphatic filariasis. Their cell wall is rich in carbohydrate sugars that expresses D-mannose, *N*-acetyl-D-glucosamine and L-fucose that can ably be recognize by MBL [164]. The low MBL expression genotype of the host was observed to increase the susceptibility to *W. bancrofti* infection in Tanzanian [165] and south-Indian [43] population. Moreover, the experimental infection of *Brugia malayi*, in Mannose-binding lectin A-deficient (MBL-$A^{-/-}$) mice have increased susceptibility to a nematode infection [164]. MBL has also been shown to interact with *Trichinella spiralis* glycoproteins and trigger the complement activation [166]. However, no genetic and/or phenotypic association studies were reported that relate MBL with Trichinosis. The rapid binding of MBL detected to the surface of *Giardia intestinalis* causing Giardiasis and the serum depleted with MBL failed to kill parasites *in vitro* [167]. Together, in line with our observations on *S. haematobium*, the higher MBL levels and genotype conferring high expression ensures the protection against the extra-cellular parasitic infections.

Leishmania donovani, an intra-cellular (macrophage) parasitic protozoan responsible for visceral leishmaniasis. Earlier reports documented that high MBL producing genotypes, alleles and high serum MBL levels were associated with active VL cases [44, 102, 104, 168]. As previous studies demonstrated, we also observed higher serum MBL levels in patients with VL. These results suggest that the high MBL levels favor the development of *L. donovani* infection and act as disease modifier in VL. Conversely, in our study the high MBL producing allele *+4Q* was observed to be a protective factor against VL. The similar discordant result was noticed in our association study of *MBL2* variants with urinary schistosomiasis where the heterozygous genotype (*+4PQ*) was observed to be a risk factor. Moreover, in contrary to previous observations, the susceptible effect −221X/Y and codon 57C was reported with cutaneous leishmaniasis caused by *Leishmania guyanensis* [169]. These contradicting observations on the impact of various *MBL2* variants need to be further explored.

Trypanosoma cruzi, an intra-cellular (fibroblast, macrophages and epithelial cells) protozoan parasite responsible for Chagas' disease (CD). MBL bound to the sugar

moieties of *T. cruzi* trypomastigotes and found to enhance the invasion process into the host cell [170]. High MBL levels were observed to associated with the risk of severe cardiomyopathy in chronic CD [171]. However, the low MBL producer *MBL2*B* genotype were present more frequently in CD patients than non-infected individuals [172]. Similar to VL, high MBL levels enhance the CD clinical outcome.

Plasmodium spp., are intra-erythrocyte living protozoan parasites responsible for malaria. The red blood cells infected with *P. falciparum* were recognized by MBL *in vitro* but not inhibited the parasite growth [173]. Low serum MBL levels and low MBL producing genotypes were associated with severe malarial anemia and parasitemia in Gabonese population [41, 174, 175]. The MBL variants were also associated of with severe malaria in Ghanian children [176] and placental malaria in primiparous Ghanian women [177]. Nevertheless, another study reported that, the MBL haplotype was associated with low-birth-weight babies, but not with placental malaria in Cameroonian women [178]. Low MBL producing variants were strongly associated with malaria in Indian population [110]. Together, MBL deficiency increases the susceptibility to malarial infections.

The role of MBL in distinct parasitic infections is controversial. The MBL deficiency and low MBL producing variants were shown to be associated with increased susceptibility to extracellular parasitic infections. However, MBL deficiency and low MBL producing variants were observed to confer protection against certain intracellular parasites such as *Trypanosoma spp*. and *Leishmania spp.* We observed a similar role of MBL in our investigated *L. donovani* infection. The widely accepted hypothesis for this differential role of MBL is that, intra cellular parasites use MBL as an opsonin to enhance their uptake into the phagocytes. This additional uptake mechanism helps the intra-cellular parasites to escape from complement mediated lysis [179]. In line, the high MBL levels was observed to enhance phagocytosis in other intracellular pathogens such as *Mycobacterium spp.,* [180]. Though, *Plasmodium spp.,* are intra-cellular parasites, they live inside of erythrocytes. Therefore, it is likely to explain that lectins do not enhance their invasion as noticed in *Leishmania spp.* and *Trypanosoma spp.* The investigations on the effect of lectins in hepatic invasion of sporozoites are limited. But,

a study reported that MBL does not alter the hepatic invasion of *P. yoelii* in MBL-A deficient mice [181]. However, the role of other lectins in enhancing the invasion of *Plasmodium spp.,* sporozoites in hepatic cells need to be further explored.

4.2 Ficolins and parasitic diseases

Ficolins are poorly investigated. In case of extra-cellular parasitic infections, the earlier study with our study samples reported that high ficolin-2 levels and high ficolin-2 producing variants were associated with protection against urinary schistosomiasis [83]. The ficolins were able to bind to the surface of *G. intestinalis* and kill parasites *in vitro* [167]. In case of intra-cellular parasitic infections, decreased ficolin-2 levels were observed in Chagas' disease. The results contradicts their previous observation on MBL in same patients [147]. However, the authors failed to justify this contrary results. The *T. cruzi* calreticulin molecule was observed to inhibit lectin pathway by direct interaction with ficolin-2 signify this lectin [30]. Nevertheless, in our investigations on VL, we noticed that high ficolin-2 levels and high ficolin-2 producing variants increase the occurrence of *L. donovani* infection. In addition, the association of *FCN2* haplotype with cutaneous leishmaniasis was reported in Syrian Arab population [182]. Ficolin-A was reported to increase the survival rate of *Plasmodium berghei* infected mice [183]. Due to the strong acute disease on admission, the ficolin-2 levels were found to be higher in severe malaria cases compared to mild malaria [184]. Based on our results, ficolins also a play a differential role in intra and extra cellular parasitic infections.

4.3 CL-K1 and parasitic diseases

No previous studies have investigated the role of CL-K1 with any infectious diseases. Though CL-K1 was reported to recognize several bacteria, fungi and viruses [31, 32], the binding to schistosomes was not demonstrated. However, we investigated CL-K1 due to fact that CL-K1 binds to the fucose rich tegument of the Schistosomes [129]. We observed that p.R216H was associated with high CL-K1 expression and schistosomiasis. Another recent study demonstrated that, three naturally occurring mutations were associated with 3MC syndrome and prevents secretion of CL-K1 from

mammalian cells and affects the normal developmental processes [185]. In agreement with MBL and ficolin-2, CL-K1 and their functional variants may be associated with protection against schistosomiasis.

4.4 MASPs and parasitic diseases

Relatively very few studies investigated MASP-2 deficiency in parasitic infections. Earlier study showed that low MASP-2 producing genotypes occurred at a higher frequency in patients with cardiomyopathy in chronic Chagas disease [186, 187]. However, experimental infection of *T. cruzi* in MASP-2-deficient mice did not confer higher susceptibility [188]. In our investigations, we noticed that high MASP-2 serum levels confer relative protection to schistosomiasis in Nigerian children. The *MASP2* p.439H variant responsible for dysfunctional MASP-2 protein was earlier reported to protect Ghanian women from placental malaria [177].

Together, we observed the differential role of lectins that depends on the parasites habitant nature either intra or extra cellular living. In addition, IL-6 was observed to regulate the lectins expression in *S. haematobium* and *L. donovani* infection. Our study observations strongly support the notion that lectins are acute phase plasma proteins [152]. In the light of our results we proposed a model (Figure 4) that illustrate the relevance of lectins in investigated parasitic diseases. In parasitic diseases caused by extra-cellular pathogens, IL-6 mediated acute-phase response of lectins may be activated. However, in spite of the up-regulation by IL-6 of APP genes, we noticed that *S. haematobium* parasite factors contribute to lower the circulating serum levels of MBL, ficolin-2 and CL-K1 by complement consumption (Figure 4A). In parasitic diseases caused by intra-cellular pathogens, IL-6 mediated acute-phase response of lectins may be activated. In turn, this favors the phagocytosis of the parasite into the phagosomes, a milieu that assists persistence of the parasite (Figure 4B). Though overlapping endemicity of schistosomiasis and visceral leishmaniasis was observed in Sudan, no data available on co-infection.

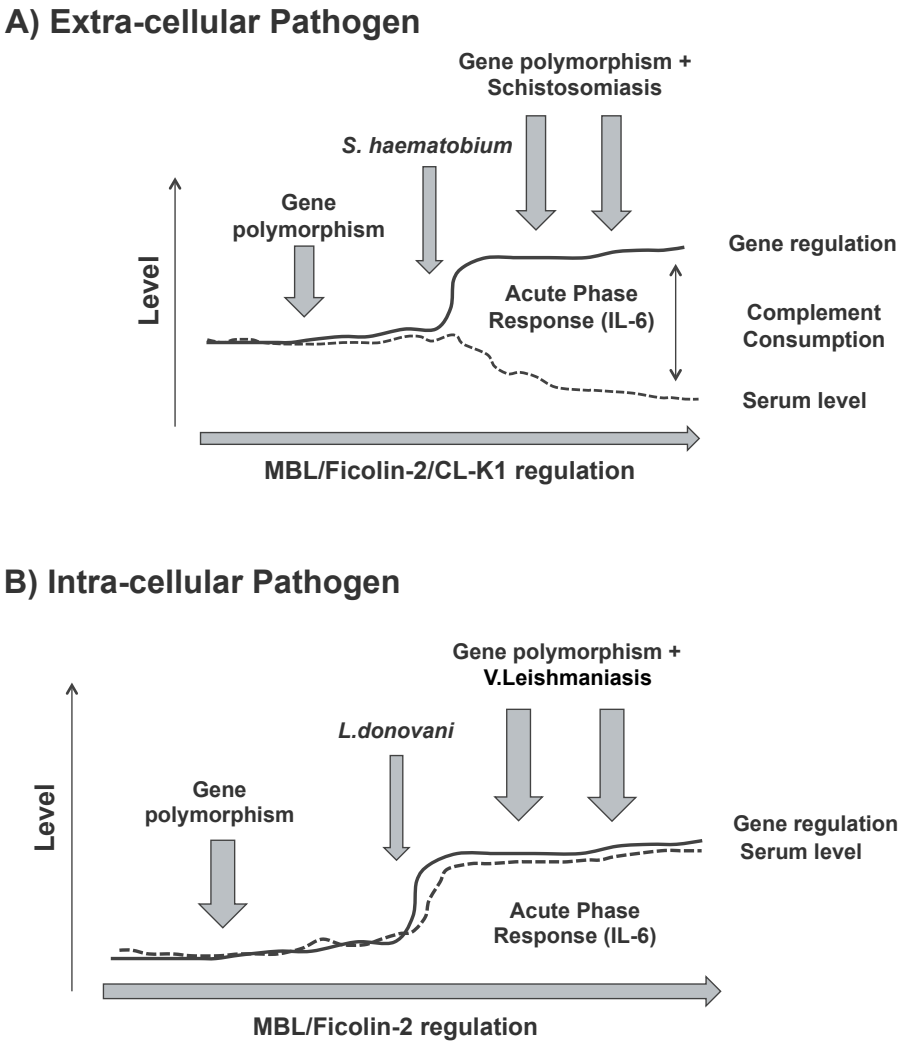

Figure 4: The model illustrates the relevance of lectins in parasitic diseases. **(A)** In parasitic diseases caused by extra-cellular pathogens, IL-6 mediated acute-phase response of lectins may be activated. However, reduced lectins serum levels noticed due to the complement consumption of the parasite. **(B)** In parasitic diseases caused by intra-cellular pathogens, IL-6 mediated acute-phase response of lectins may be activated. In turn, this favors the phagocytosis of the parasite into the phagosomes, a milieu that assists persistence of the parasite.

However, it is observed that *S. mansoni* delays the healing of cutaneous lesions caused by *L. major* in co-infected mice [189]. The intestinal helminth infections was observed to influence clinical outcome of cutaneous leishmaniasis in patients [190]. Our observations warrant further experimental and clinical investigations of lectins on co-infection of intra and extracellular parasitic infections.

Conclusion

The impact of lectins deficiency and its association to parasitic diseases has been previously investigated. However, the scenario is highly debated as the studies suggest a positive or negative impact of lectins deficiency on susceptibility to parasitic infections based on their cellular habitants. In this thesis, we investigated the interaction of lectins with intra and extra cellular parasites, as it is important to understand the host-pathogen interactions to eradicate the disease. Our findings support the observation that in extra cellular *S. haematobium* infection, MBL, Collectin-11 (CL-K1), MASP-2 and their functional variants were associated with relative protection in a Nigerian study group. Dissimilar, in intra-cellular *L. donovani* infection, MBL, ficolin-2 and their functional variants were observed to be a susceptible host factor. Here we conclude based on the observations that lectins play a differential role during intra and extra-cellular parasitic infections and serve as a ‚double-edged sword'. Our findings suggest that parasite cellular habitat determines the role of lectins. We also observed that the lectins expression was regulated by IL-6 in investigated parasitic infections. Taken together, these investigations lay a basis to understand the role of lectins in intra and extracellular parasitic infections.

References

1. Schneider DS, Ayres JS: **Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases**. *Nature reviews Immunology* 2008, **8**(11):889-895.
2. Dranoff G: **Cytokines in cancer pathogenesis and cancer therapy**. *Nature reviews Cancer* 2004, **4**(1):11-22.
3. Dunkelberger JR, Song WC: **Complement and its role in innate and adaptive immune responses**. *Cell research* 2010, **20**(1):34-50.
4. Beutler B: **Innate immunity: an overview**. *Molecular immunology* 2004, **40**(12):845-859.
5. Song WC, Sarrias MR, Lambris JD: **Complement and innate immunity**. *Immunopharmacology* 2000, **49**(1-2):187-198.
6. Carroll MC: **The complement system in regulation of adaptive immunity**. *Nature immunology* 2004, **5**(10):981-986.
7. Song WC: **Crosstalk between complement and toll-like receptors**. *Toxicologic pathology* 2012, **40**(2):174-182.
8. Wallis R: **Interactions between mannose-binding lectin and MASPs during complement activation by the lectin pathway**. *Immunobiology* 2007, **212**(4-5):289-299.
9. Tegla CA, Cudrici C, Patel S, Trippe R, 3rd, Rus V, Niculescu F, Rus H: **Membrane attack by complement: the assembly and biology of terminal complement complexes**. *Immunologic research* 2011, **51**(1):45-60.
10. Cestari I, Evans-Osses I, Schlapbach LJ, de Messias-Reason I, Ramirez MI: **Mechanisms of complement lectin pathway activation and resistance by trypanosomatid parasites**. *Molecular immunology* 2013, **53**(4):328-334.
11. Fujita T: **Evolution of the lectin-complement pathway and its role in innate immunity**. *Nature reviews Immunology* 2002, **2**(5):346-353.
12. Botto M, Kirschfink M, Macor P, Pickering MC, Wurzner R, Tedesco F: **Complement in human diseases: Lessons from complement deficiencies**. *Molecular immunology* 2009, **46**(14):2774-2783.
13. Matsushita M, Endo Y, Hamasaki N, Fujita T: **Activation of the lectin complement pathway by ficolins**. *International immunopharmacology* 2001, **1**(3):359-363.
14. Heitzeneder S, Seidel M, Forster-Waldl E, Heitger A: **Mannan-binding lectin deficiency - Good news, bad news, doesn't matter?** *Clinical immunology* 2012, **143**(1):22-38.
15. Guo N, Mogues T, Weremowicz S, Morton CC, Sastry KN: **The human ortholog of rhesus mannose-binding protein-A gene is an expressed pseudogene that localizes to chromosome 10**. *Mamm Genome* 1998, **9**(3):246-249.
16. Garred P, Larsen F, Seyfarth J, Fujita R, Madsen HO: **Mannose-binding lectin and its genetic variants**. *Genes and immunity* 2006, **7**(2):85-94.
17. Garred P, Larsen F, Madsen HO, Koch C: **Mannose-binding lectin deficiency--revisited**. *Molecular immunology* 2003, **40**(2-4):73-84.
18. Garred P: **Mannose-binding lectin genetics: from A to Z**. *Biochem Soc Trans* 2008, **36**(Pt 6):1461-1466.
19. Madsen HO, Satz ML, Hogh B, Svejgaard A, Garred P: **Different molecular events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America**. *Journal of immunology* 1998, **161**(6):3169-3175.
20. Bernig T, Breunis W, Brouwer N, Hutchinson A, Welch R, Roos D, Kuijpers T, Chanock S: **An analysis of genetic variation across the MBL2 locus in Dutch Caucasians indicates that 3'**

haplotypes could modify circulating levels of mannose-binding lectin. *Hum Genet* 2005, **118**(3-4):404-415.

21. Jack DL, Klein NJ, Turner MW: **Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis**. *Immunol Rev* 2001, **180**:86-99.
22. Eisen DP, Minchinton RM: **Impact of mannose-binding lectin on susceptibility to infectious diseases**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2003, **37**(11):1496-1505.
23. Mason CP, Tarr AW: **Human lectins and their roles in viral infections**. *Molecules* 2015, **20**(2):2229-2271.
24. Mayilyan KR: **Complement genetics, deficiencies, and disease associations**. *Protein Cell* 2012, **3**(7):487-496.
25. Matsushita M: **Ficolins in complement activation**. *Molecular immunology* 2013, **55**(1):22-26.
26. Teh C, Le Y, Lee SH, Lu J: **M-ficolin is expressed on monocytes and is a lectin binding to N-acetyl-D-glucosamine and mediates monocyte adhesion and phagocytosis of Escherichia coli**. *Immunology* 2000, **101**(2):225-232.
27. Gout E, Garlatti V, Smith DF, Lacroix M, Dumestre-Perard C, Lunardi T, Martin L, Cesbron JY, Arlaud GJ, Gaboriaud C *et al*: **Carbohydrate recognition properties of human ficolins: glycan array screening reveals the sialic acid binding specificity of M-ficolin**. *The Journal of biological chemistry* 2010, **285**(9):6612-6622.
28. Ren Y, Ding Q, Zhang X: **Ficolins and infectious diseases**. *Virologica Sinica* 2014, **29**(1):25-32.
29. Swierzko AS, Atkinson AP, Cedzynski M, Macdonald SL, Szala A, Domzalska-Popadiuk I, Borkowska-Klos M, Jopek A, Szczapa J, Matsushita M *et al*: **Two factors of the lectin pathway of complement, l-ficolin and mannan-binding lectin, and their associations with prematurity, low birthweight and infections in a large cohort of Polish neonates**. *Molecular immunology* 2009, **46**(4):551-558.
30. Sosoniuk E, Vallejos G, Kenawy H, Gaboriaud C, Thielens N, Fujita T, Schwaeble W, Ferreira A, Valck C: **Trypanosoma cruzi calreticulin inhibits the complement lectin pathway activation by direct interaction with L-Ficolin**. *Molecular immunology* 2014, **60**(1):80-85.
31. Keshi H, Sakamoto T, Kawai T, Ohtani K, Katoh T, Jang SJ, Motomura W, Yoshizaki T, Fukuda M, Koyama S *et al*: **Identification and characterization of a novel human collectin CL-K1**. *Microbiology and immunology* 2006, **50**(12):1001-1013.
32. Hansen S, Selman L, Palaniyar N, Ziegler K, Brandt J, Kliem A, Jonasson M, Skjoedt MO, Nielsen O, Hartshorn K *et al*: **Collectin 11 (CL-11, CL-K1) is a MASP-1/3-associated plasma collectin with microbial-binding activity**. *Journal of immunology* 2010, **185**(10):6096-6104.
33. Rooryck C, Diaz-Font A, Osborn DP, Chabchoub E, Hernandez-Hernandez V, Shamseldin H, Kenny J, Waters A, Jenkins D, Kaissi AA *et al*: **Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome**. *Nature genetics* 2011, **43**(3):197-203.
34. Henriksen ML, Brandt J, Andrieu JP, Nielsen C, Jensen PH, Holmskov U, Jorgensen TJ, Palarasah Y, Thielens NM, Hansen S: **Heteromeric complexes of native collectin kidney 1 and collectin liver 1 are found in the circulation with MASPs and activate the complement system**. *Journal of immunology* 2013, **191**(12):6117-6127.
35. Holmskov U, Thiel S, Jensenius JC: **Collections and ficolins: humoral lectins of the innate immune defense**. *Annual review of immunology* 2003, **21**:547-578.
36. Boldt AB, Grisbach C, Steffensen R, Thiel S, Kun JF, Jensenius JC, Messias-Reason IJ: **Multiplex sequence-specific polymerase chain reaction reveals new MASP2 haplotypes associated with MASP-2 and MAp19 serum levels**. *Hum Immunol* 2011, **72**(9):753-760.

37. Thiel S, Kolev M, Degn S, Steffensen R, Hansen AG, Ruseva M, Jensenius JC: **Polymorphisms in mannan-binding lectin (MBL)-associated serine protease 2 affect stability, binding to MBL, and enzymatic activity**. *Journal of immunology* 2009, **182**(5):2939-2947.
38. Thiel S, Steffensen R, Christensen IJ, Ip WK, Lau YL, Reason IJ, Eiberg H, Gadjeva M, Ruseva M, Jensenius JC: **Deficiency of mannan-binding lectin associated serine protease-2 due to missense polymorphisms**. *Genes and immunity* 2007, **8**(2):154-163.
39. Olszowski T, Poziomkowska-Gesicka I, Jensenius JC, Adler G: **Lectin pathway of complement activation in a Polish woman with MASP-2 deficiency**. *Immunobiology* 2014, **219**(4):261-262.
40. Beltrame MH, Boldt AB, Catarino SJ, Mendes HC, Boschmann SE, Goeldner I, Messias-Reason I: **MBL-associated serine proteases (MASPs) and infectious diseases**. *Molecular immunology* 2015, **67**(1):85-100.
41. Boldt AB, Messias-Reason IJ, Lell B, Issifou S, Pedroso ML, Kremsner PG, Kun JF: **Haplotype specific-sequencing reveals MBL2 association with asymptomatic Plasmodium falciparum infection**. *Malar J* 2009, **8**:97.
42. Eisen DP, Dean MM, Boermeester MA, Fidler KJ, Gordon AC, Kronborg G, Kun JF, Lau YL, Payeras A, Valdimarsson H *et al*: **Low serum mannose-binding lectin level increases the risk of death due to pneumococcal infection**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2008, **47**(4):510-516.
43. Choi EH, Zimmerman PA, Foster CB, Zhu S, Kumaraswami V, Nutman TB, Chanock SJ: **Genetic polymorphisms in molecules of innate immunity and susceptibility to infection with Wuchereria bancrofti in South India**. *Genes and immunity* 2001, **2**(5):248-253.
44. Alonso DP, Ferreira AF, Ribolla PE, de Miranda Santos IK, do Socorro Pires e Cruz M, Aecio de Carvalho F, Abatepaulo AR, Lamounier Costa D, Werneck GL, Farias TJ *et al*: **Genotypes of the mannan-binding lectin gene and susceptibility to visceral leishmaniasis and clinical complications**. *The Journal of infectious diseases* 2007, **195**(8):1212-1217.
45. Hoal-Van Helden EG, Epstein J, Victor TC, Hon D, Lewis LA, Beyers N, Zurakowski D, Ezekowitz AB, Van Helden PD: **Mannose-binding protein B allele confers protection against tuberculous meningitis**. *Pediatr Res* 1999, **45**(4 Pt 1):459-464.
46. de Messias-Reason IJ, Boldt AB, Moraes Braga AC, Von Rosen Seeling Stahlke E, Dornelles L, Pereira-Ferrari L, Kremsner PG, Kun JF: **The association between mannan-binding lectin gene polymorphism and clinical leprosy: new insight into an old paradigm**. *The Journal of infectious diseases* 2007, **196**(9):1379-1385.
47. Denholm JT, McBryde ES, Eisen DP: **Mannose-binding lectin and susceptibility to tuberculosis: a meta-analysis**. *Clinical and experimental immunology* 2010, **162**(1):84-90.
48. Luo F, Sun X, Wang Y, Wang Q, Wu Y, Pan Q, Fang C, Zhang XL: **Ficolin-2 defends against virulent Mycobacteria tuberculosis infection in vivo, and its insufficiency is associated with infection in humans**. *PloS one* 2013, **8**(9):e73859.
49. Boldt AB, Goeldner I, Stahlke ER, Thiel S, Jensenius JC, de Messias-Reason IJ: **Leprosy association with low MASP-2 levels generated by MASP2 haplotypes and polymorphisms flanking MAp19 exon 5**. *PloS one* 2013, **8**(7):e69054.
50. Goto H, Sanchez MCA: **Does the Complement System Work for or Against the Host during Parasite Infections**. *International Trends in Immunity* 2013, **1**:11-23.
51. Yazdanbakhsh M, Sacks DL: **Why does immunity to parasites take so long to develop?** *Nature reviews Immunology* 2010, **10**(2):80-81.
52. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY *et al*: **Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010**. *Lancet* 2012, **380**(9859):2095-2128.

53. Andrews KT, Fisher G, Skinner-Adams TS: **Drug repurposing and human parasitic protozoan diseases**. *International journal for parasitology Drugs and drug resistance* 2014, **4**(2):95-111.
54. King CH: **Parasites and poverty: the case of schistosomiasis**. *Acta tropica* 2010, **113**(2):95-104.
55. Luo S, Skerka C, Kurzai O, Zipfel PF: **Complement and innate immune evasion strategies of the human pathogenic fungus Candida albicans**. *Molecular immunology* 2013, **56**(3):161-169.
56. Cestari Idos S, Krarup A, Sim RB, Inal JM, Ramirez MI: **Role of early lectin pathway activation in the complement-mediated killing of Trypanosoma cruzi**. *Molecular immunology* 2009, **47**(2-3):426-437.
57. Garred P, Nielsen MA, Kurtzhals JA, Malhotra R, Madsen HO, Goka BQ, Akanmori BD, Sim RB, Hviid L: **Mannose-binding lectin is a disease modifier in clinical malaria and may function as opsonin for Plasmodium falciparum-infected erythrocytes**. *Infection and immunity* 2003, **71**(9):5245-5253.
58. Green PJ, Feizi T, Stoll MS, Thiel S, Prescott A, McConville MJ: **Recognition of the major cell surface glycoconjugates of Leishmania parasites by the human serum mannan-binding protein**. *Molecular and biochemical parasitology* 1994, **66**(2):319-328.
59. Klabunde J, Berger J, Jensenius JC, Klinkert MQ, Zelck UE, Kremsner PG, Kun JF: **Schistosoma mansoni: adhesion of mannan-binding lectin to surface glycoproteins of cercariae and adult worms**. *Experimental parasitology* 2000, **95**(4):231-239.
60. Brittingham A, Morrison CJ, McMaster WR, McGwire BS, Chang KP, Mosser DM: **Role of the Leishmania surface protease gp63 in complement fixation, cell adhesion, and resistance to complement-mediated lysis**. *Journal of immunology* 1995, **155**(6):3102-3111.
61. Conroy AL, Silver KL, Zhong K, Rennie M, Ward P, Sarma JV, Molyneux ME, Sled J, Fletcher JF, Rogerson S *et al*: **Complement activation and the resulting placental vascular insufficiency drives fetal growth restriction associated with placental malaria**. *Cell Host Microbe* 2013, **13**(2):215-226.
62. Kim H, Erdman LK, Lu Z, Serghides L, Zhong K, Dhabangi A, Musoke C, Gerard C, Cserti-Gazdewich C, Liles WC *et al*: **Functional roles for C5a and C5aR but not C5L2 in the pathogenesis of human and experimental cerebral malaria**. *Infection and immunity* 2014, **82**(1):371-379.
63. Jenkins-Holick DS, Kaul TL: **Schistosomiasis**. *Urologic nursing* 2013, **33**(4):163-170.
64. Organization WH: **Schistosomiasis Fact sheet-Number 115**. In.; 2014.
65. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J: **Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk**. *The Lancet infectious diseases* 2006, **6**(7):411-425.
66. Hotez PJ, Kamath A: **Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden**. *PLoS neglected tropical diseases* 2009, **3**(8):e412.
67. Bamgbola OF: **Urinary schistosomiasis**. *Pediatric nephrology* 2014.
68. Hotez PJ, Fenwick A, Kjetland EF: **Africa's 32 cents solution for HIV/AIDS**. *PLoS neglected tropical diseases* 2009, **3**(5):e430.
69. Secor WE: **The effects of schistosomiasis on HIV/AIDS infection, progression and transmission**. *Current opinion in HIV and AIDS* 2012, **7**(3):254-259.
70. Gryseels B, Polman K, Clerinx J, Kestens L: **Human schistosomiasis**. *Lancet* 2006, **368**(9541):1106-1118.
71. van der Werf MJ, de Vlas SJ, Brooker S, Looman CW, Nagelkerke NJ, Habbema JD, Engels D: **Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa**. *Acta tropica* 2003, **86**(2-3):125-139.

72. Mbabazi PS, Andan O, Fitzgerald DW, Chitsulo L, Engels D, Downs JA: **Examining the relationship between urogenital schistosomiasis and HIV infection**. *PLoS neglected tropical diseases* 2011, **5**(12):e1396.
73. Kjetland EF, Ndhlovu PD, Gomo E, Mduluza T, Midzi N, Gwanzura L, Mason PR, Sandvik L, Friis H, Gundersen SG: **Association between genital schistosomiasis and HIV in rural Zimbabwean women**. *Aids* 2006, **20**(4):593-600.
74. Hotez P, Whitham M: **Helminth infections: a new global women's health agenda**. *Obstetrics and gynecology* 2014, **123**(1):155-160.
75. Herrera LA, Benitez-Bribiesca L, Mohar A, Ostrosky-Wegman P: **Role of infectious diseases in human carcinogenesis**. *Environmental and molecular mutagenesis* 2005, **45**(2-3):284-303.
76. Shaker OG, Hammam OA, El Leithy TR, El Ganzoury H, Wishahi MM, Mikhailidis DP: **Molecular markers and bladder carcinoma: Schistosomal and non-schistosomal**. *Clinical biochemistry* 2011, **44**(2-3):237-244.
77. Vennervald BJ, Polman K: **Helminths and malignancy**. *Parasite immunology* 2009, **31**(11):686-696.
78. Colley DG, Bustinduy AL, Secor WE, King CH: **Human schistosomiasis**. *Lancet* 2014, **383**(9936):2253-2264.
79. Stothard JR, Sousa-Figueiredo JC, Betson M, Bustinduy A, Reinhard-Rupp J: **Schistosomiasis in African infants and preschool children: let them now be treated!** *Trends in parasitology* 2013, **29**(4):197-205.
80. Cummings RD, Nyame AK: **Schistosome glysoconjugates**. *Biochim Biophys Acta* 1999, **1455**(2-3):363-374.
81. Hayunga EG, Sumner MP: **Expression of lectin-binding surface glycoproteins during the development of Schistosoma mansoni schistosomula**. *J Parasitol* 1986, **72**(6):913-920.
82. Schmidt J: **Glycans with N-acetyllactosamine type 2-like residues covering adult Schistosoma mansoni, and glycomimesis as a putative mechanism of immune evasion**. *Parasitology* 1995, **111 (Pt 3)**:325-336.
83. Ouf EA, Ojurongbe O, Akindele AA, Sina-Agbaje OR, Van Tong H, Adeyeba AO, Kremsner PG, Kun JF, Velavan T: **Ficolin-2 levels and FCN2 genetic polymorphisms as a susceptibility factor in schistosomiasis**. *The Journal of infectious diseases* 2012, **206**(4):562-570.
84. Herwaldt BL: **Leishmaniasis**. *Lancet* 1999, **354**(9185):1191-1199.
85. Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, Alvar J, Boelaert M: **Visceral leishmaniasis: what are the needs for diagnosis, treatment and control?** *Nature reviews Microbiology* 2007, **5**(11):873-882.
86. Alvar J, Yactayo S, Bern C: **Leishmaniasis and poverty**. *Trends in parasitology* 2006, **22**(12):552-557.
87. Organization WH: **Leishmaniasis Fact Sheet-Number 375**. In.; 2015.
88. Desjeux P: **Leishmaniasis. Public health aspects and control**. *Clinics in dermatology* 1996, **14**(5):417-423.
89. Lukes J, Mauricio IL, Schonian G, Dujardin JC, Soteriadou K, Dedet JP, Kuhls K, Tintaya KW, Jirku M, Chocholova E *et al*: **Evolutionary and geographical history of the Leishmania donovani complex with a revision of current taxonomy**. *Proceedings of the National Academy of Sciences of the United States of America* 2007, **104**(22):9375-9380.
90. Mueller YK, Kolaczinski JH, Koech T, Lokwang P, Riongoita M, Velilla E, Brooker SJ, Chappuis F: **Clinical epidemiology, diagnosis and treatment of visceral leishmaniasis in the Pokot endemic area of Uganda and Kenya**. *The American journal of tropical medicine and hygiene* 2014, **90**(1):33-39.

91. Desjeux P: **Leishmaniasis: current situation and new perspectives**. *Comparative immunology, microbiology and infectious diseases* 2004, **27**(5):305-318.
92. Mathers CD, Ezzati M, Lopez AD: **Measuring the burden of neglected tropical diseases: the global burden of disease framework**. *PLoS neglected tropical diseases* 2007, **1**(2):e114.
93. Mukhopadhyay D, Dalton JE, Kaye PM, Chatterjee M: **Post kala-azar dermal leishmaniasis: an unresolved mystery**. *Trends in parasitology* 2014, **30**(2):65-74.
94. Olliaro PL, Guerin PJ, Gerstl S, Haaskjold AA, Rottingen JA, Sundar S: **Treatment options for visceral leishmaniasis: a systematic review of clinical studies done in India, 1980-2004**. *The Lancet infectious diseases* 2005, **5**(12):763-774.
95. Thakur CP: **Socio-economics of visceral leishmaniasis in Bihar (India)**. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2000, **94**(2):156-157.
96. van Griensven J: **Editorial Commentary: Visceral Leishmaniasis and HIV Coinfection in Bihar, India: A Wake-up Call?** *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014, **59**(4):556-558.
97. Murray HW, Berman JD, Davies CR, Saravia NG: **Advances in leishmaniasis**. *Lancet* 2005, **366**(9496):1561-1577.
98. Muniaraj M: **The lost hope of elimination of Kala-azar (visceral leishmaniasis) by 2010 and cyclic occurrence of its outbreak in India, blame falls on vector control practices or co-infection with human immunodeficiency virus or therapeutic modalities?** *Tropical parasitology* 2014, **4**(1):10-19.
99. Hommel M: **Visceral leishmaniasis: biology of the parasite**. *The Journal of infection* 1999, **39**(2):101-111.
100. Moradin N, Descoteaux A: **Leishmania promastigotes: building a safe niche within macrophages**. *Frontiers in cellular and infection microbiology* 2012, **2**:121.
101. Ambrosio AR, De Messias-Reason IJ: **Leishmania (Viannia) braziliensis: interaction of mannose-binding lectin with surface glycoconjugates and complement activation. An antibody-independent defence mechanism**. *Parasite immunology* 2005, **27**(9):333-340.
102. Santos IK, Costa CH, Krieger H, Feitosa MF, Zurakowski D, Fardin B, Gomes RB, Weiner DL, Harn DA, Ezekowitz RA *et al*: **Mannan-binding lectin enhances susceptibility to visceral leishmaniasis**. *Infection and immunity* 2001, **69**(8):5212-5215.
103. Alonso DP, Ferreira AF, Ribolla PE, de Miranda Santos IK, do Socorro Pires e C, Aecio de CF, Abatepaulo AR, Lamounier CD, Werneck GL, Farias TJ *et al*: **Genotypes of the mannan-binding lectin gene and susceptibility to visceral leishmaniasis and clinical complications**. *JInfectDis* 2007, **195**(8):1212-1217.
104. Hamdi S, Ejghal R, Idrissi M, Ezzikouri S, Hida M, Soong L, Amarouch H, Lemrani M: **A variant in the promoter of MBL2 is associated with protection against visceral leishmaniasis in Morocco**. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases* 2013, **13**:162-167.
105. Bode JG, Albrecht U, Haussinger D, Heinrich PC, Schaper F: **Hepatic acute phase proteins--regulation by IL-6- and IL-1-type cytokines involving STAT3 and its crosstalk with NF-kappaB-dependent signaling**. *EurJCell Biol* 2012, **91**(6-7):496-505.
106. Dalmon J, Laurent M, Courtois G: **The human beta fibrinogen promoter contains a hepatocyte nuclear factor 1-dependent interleukin-6-responsive element**. *MolCell Biol* 1993, **13**(2):1183-1193.
107. Arai T, Tabona P, Summerfield JA: **Human mannose-binding protein gene is regulated by interleukins, dexamethasone and heat shock**. *QJMed* 1993, **86**(9):575-582.

108. Costa DL, Rocha RL, Carvalho RM, Lima-Neto AS, Harhay MO, Costa CH, Barral-Neto M, Barral AP: **Serum cytokines associated with severity and complications of kala-azar**. *PathogGlobHealth* 2013, **107**(2):78-87.
109. Goeldner I, Skare TL, Utiyama SR, Nisihara RM, Tong H, Messias-Reason IJ, Velavan TP: **Mannose binding lectin and susceptibility to rheumatoid arthritis in Brazilian patients and their relatives**. *PLoSOne* 2014, **9**(4):e95519.
110. Jha AN, Sundaravadivel P, Singh VK, Pati SS, Patra PK, Kremsner PG, Velavan TP, Singh L, Thangaraj K: **MBL2 variations and malaria susceptibility in Indian populations**. *Infection and immunity* 2014, **82**(1):52-61.
111. La Flamme AC, MacDonald AS, Pearce EJ: **Role of IL-6 in directing the initial immune response to schistosome eggs**. *Journal of immunology* 2000, **164**(5):2419-2426.
112. Jenkins SJ, Hewitson JP, Ferret-Bernard S, Mountford AP: **Schistosome larvae stimulate macrophage cytokine production through TLR4-dependent and -independent pathways**. *IntImmunol* 2005, **17**(11):1409-1418.
113. Graham BB, Chabon J, Kumar R, Kolosionek E, Gebreab L, Debella E, Edwards M, Diener K, Shade T, Bifeng G *et al*: **Protective role of IL-6 in vascular remodeling in Schistosoma pulmonary hypertension**. *American journal of respiratory cell and molecular biology* 2013, **49**(6):951-959.
114. Islamuddin M, Chouhan G, Farooque A, Dwarakanath BS, Sahal D, Afrin F: **Th1-biased immunomodulation and therapeutic potential of Artemisia annua in murine visceral leishmaniasis**. *PLoSNeglTropDis* 2015, **9**(1):e3321.
115. Ansari NA, Saluja S, Salotra P: **Elevated levels of interferon-gamma, interleukin-10, and interleukin-6 during active disease in Indian kala azar**. *ClinImmunol* 2006, **119**(3):339-345.
116. van der Poll T, Zijlstra EE, Mevissen M: **Interleukin 6 during active visceral leishmaniasis and after treatment**. *ClinImmunolImmunopathol* 1995, **77**(1):111-114.
117. Hatzigeorgiou DE, He S, Sobel J, Grabstein KH, Hafner A, Ho JL: **IL-6 down-modulates the cytokine-enhanced antileishmanial activity in human macrophages**. *JImmunol* 1993, **151**(7):3682-3692.
118. Pettigrew HD, Teuber SS, Gershwin ME: **Clinical significance of complement deficiencies**. *Annals of the New York Academy of Sciences* 2009, **1173**:108-123.
119. Joiner KA: **Complement evasion by bacteria and parasites**. *Annu Rev Microbiol* 1988, **42**:201-230.
120. Ricklin D, Hajishengallis G, Yang K, Lambris JD: **Complement: a key system for immune surveillance and homeostasis**. *Nature immunology* 2010, **11**(9):785-797.
121. Dessein AJ, Couissinier P, Demeure C, Rihet P, Kohlstaedt S, Carneiro-Carvalho D, Ouattara M, Goudot-Crozel V, Dessein H, Bourgois A *et al*: **Environmental, genetic and immunological factors in human resistance to Schistosoma mansoni**. *Immunological investigations* 1992, **21**(5):423-453.
122. Abel L, Dessein A: **Genetic predisposition to high infections in an endemic area of Schistosoma mansoni**. *Revista da Sociedade Brasileira de Medicina Tropical* 1991, **24**(1):1-3.
123. Butterworth AE, Capron M, Cordingley JS, Dalton PR, Dunne DW, Kariuki HC, Kimani G, Koech D, Mugambi M, Ouma JH *et al*: **Immunity after treatment of human schistosomiasis mansoni. II. Identification of resistant individuals, and analysis of their immune responses**. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1985, **79**(3):393-408.
124. Marquet S, Abel L, Hillaire D, Dessein H, Kalil J, Feingold J, Weissenbach J, Dessein AJ: **Genetic localization of a locus controlling the intensity of infection by Schistosoma mansoni on chromosome 5q31-q33**. *Nature genetics* 1996, **14**(2):181-184.
125. Muller-Myhsok B, Stelma FF, Guisse-Sow F, Muntau B, Thye T, Burchard GD, Gryseels B, Horstmann RD: **Further evidence suggesting the presence of a locus, on human chromosome**

5q31-q33, influencing the intensity of infection with Schistosoma mansoni. *American journal of human genetics* 1997, **61**(2):452-454.
126. Quinnell RJ: **Genetics of susceptibility to human helminth infection**. *International journal for parasitology* 2003, **33**(11):1219-1231.
127. Mullighan CG, Heatley S, Doherty K, Szabo F, Grigg A, Hughes TP, Schwarer AP, Szer J, Tait BD, Bik To L *et al*: **Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation**. *Blood* 2002, **99**(10):3524-3529.
128. Tao R, Hua CZ, Hu YZ, Shang SQ: **Genetic polymorphisms and serum levels of mannose-binding lectin in Chinese pediatric patients with common infectious diseases**. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* 2012, **16**(5):e403-407.
129. Selman L, Hansen S: **Structure and function of collectin liver 1 (CL-L1) and collectin 11 (CL-11, CL-K1)**. *Immunobiology* 2012, **217**(9):851-863.
130. Peterson NA, Hokke CH, Deelder AM, Yoshino TP: **Glycotope analysis in miracidia and primary sporocysts of Schistosoma mansoni: differential expression during the miracidium-to-sporocyst transformation**. *International journal for parasitology* 2009, **39**(12):1331-1344.
131. International HapMap C: **A haplotype map of the human genome**. *Nature* 2005, **437**(7063):1299-1320.
132. Xue Y, Zhang X, Huang N, Daly A, Gillson CJ, Macarthur DG, Yngvadottir B, Nica AC, Woodwark C, Chen Y *et al*: **Population differentiation as an indicator of recent positive selection in humans: an empirical evaluation**. *Genetics* 2009, **183**(3):1065-1077.
133. Sorensen R, Thiel S, Jensenius JC: **Mannan-binding-lectin-associated serine proteases, characteristics and disease associations**. *Springer seminars in immunopathology* 2005, **27**(3):299-319.
134. Fakeye O, Fagbule D: **Age and anthropometric status of Nigerian girls at puberty: implication for the introduction of sex education into secondary schools**. *West African journal of medicine* 1990, **9**(3):226-231.
135. Milner T, Reilly L, Nausch N, Midzi N, Mduluza T, Maizels R, Mutapi F: **Circulating cytokine levels and antibody responses to human Schistosoma haematobium: IL-5 and IL-10 levels depend upon age and infection status**. *Parasite immunology* 2010, **32**(11-12):710-721.
136. Mutapi F, Winborn G, Midzi N, Taylor M, Mduluza T, Maizels RM: **Cytokine responses to Schistosoma haematobium in a Zimbabwean population: contrasting profiles for IFN-gamma, IL-4, IL-5 and IL-10 with age**. *BMC infectious diseases* 2007, **7**:139.
137. Nausch N, Louis D, Lantz O, Peguillet I, Trottein F, Chen IY, Appleby LJ, Bourke CD, Midzi N, Mduluza T *et al*: **Age-related patterns in human myeloid dendritic cell populations in people exposed to Schistosoma haematobium infection**. *PLoS neglected tropical diseases* 2012, **6**(9):e1824.
138. Yole DS, Gikuru SK, Wango EO, Kithome K, Kiarie S, Limo M: **Influence of age of mice on the susceptibility to murine schistosomiasis infection**. *African journal of health sciences* 2006, **13**(1-2):47-54.
139. Warren KS: **Regulation of the prevalence and intensity of schistosomiasis in man: immunology or ecology?** *The Journal of infectious diseases* 1973, **127**(5):595-609.
140. Fulford AJ, Webster M, Ouma JH, Kimani G, Dunne DW: **Puberty and Age-related Changes in Susceptibility to Schistosome Infection**. *Parasitology today* 1998, **14**(1):23-26.
141. McCall LI, Zhang WW, Matlashewski G: **Determinants for the development of visceral leishmaniasis disease**. *PLoS pathogens* 2013, **9**(1):e1003053.

142. Blackwell JM, Fakiola M, Ibrahim ME, Jamieson SE, Jeronimo SB, Miller EN, Mishra A, Mohamed HS, Peacock CS, Raju M *et al*: **Genetics and visceral leishmaniasis: of mice and man**. *Parasite immunology* 2009, **31**(5):254-266.
143. Leish GENC, Wellcome Trust Case Control C, Fakiola M, Strange A, Cordell HJ, Miller EN, Pirinen M, Su Z, Mishra A, Mehrotra S *et al*: **Common variants in the HLA-DRB1-HLA-DQA1 HLA class II region are associated with susceptibility to visceral leishmaniasis**. *Nature genetics* 2013, **45**(2):208-213.
144. Sakthianandeswaren A, Foote SJ, Handman E: **The role of host genetics in leishmaniasis**. *Trends in parasitology* 2009, **25**(8):383-391.
145. Brittingham A, Mosser DM: **Exploitation of the complement system by Leishmania promastigotes**. *Parasitology today* 1996, **12**(11):444-447.
146. Krarup A, Thiel S, Hansen A, Fujita T, Jensenius JC: **L-ficolin is a pattern recognition molecule specific for acetyl groups**. *The Journal of biological chemistry* 2004, **279**(46):47513-47519.
147. Luz PR, Boldt AB, Grisbach C, Kun JF, Velavan TP, Messias-Reason IJ: **Association of L-ficolin levels and FCN2 genotypes with chronic Chagas disease**. *PloS one* 2013, **8**(4):e60237.
148. Cedzynski M, Nuytinck L, Atkinson AP, St Swierzko A, Zeman K, Szemraj J, Szala A, Turner ML, Kilpatrick DC: **Extremes of L-ficolin concentration in children with recurrent infections are associated with single nucleotide polymorphisms in the FCN2 gene**. *Clinical and experimental immunology* 2007, **150**(1):99-104.
149. Hummelshoj T, Munthe-Fog L, Madsen HO, Garred P: **Functional SNPs in the human ficolin (FCN) genes reveal distinct geographical patterns**. *Molecular immunology* 2008, **45**(9):2508-2520.
150. Gauldie J, Lamontagne L, Stadnyk A: **Acute phase response in infectious disease**. *SurvSynthPatholRes* 1985, **4**(2):126-151.
151. Pavare J, Grope I, Kalnins I, Gardovska D: **High-mobility group box-1 protein, lipopolysaccharide-binding protein, interleukin-6 and C-reactive protein in children with community acquired infections and bacteraemia: a prospective study**. *BMCInfectDis* 2010, **10**:28.
152. Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC: **The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response**. *Clinical and experimental immunology* 1992, **90**(1):31-35.
153. Fraser DA, Bohlson SS, Jasinskiene N, Rawal N, Palmarini G, Ruiz S, Rochford R, Tenner AJ: **C1q and MBL, components of the innate immune system, influence monocyte cytokine expression**. *Journal of leukocyte biology* 2006, **80**(1):107-116.
154. McDonald EA, Pond-Tor S, Jarilla B, Sagliba MJ, Gonzal A, Amoylen AJ, Olveda R, Acosta L, Gundogan F, Ganley-Leal LM *et al*: **Schistosomiasis japonica during pregnancy is associated with elevated endotoxin levels in maternal and placental compartments**. *The Journal of infectious diseases* 2014, **209**(3):468-472.
155. El-Salahy EM: **Evaluation of cytokeratin-19 & cytokeratin-20 and interleukin-6 in Egyptian bladder cancer patients**. *Clinical biochemistry* 2002, **35**(8):607-613.
156. Pancre V, Monte D, Delanoye A, Capron A, Auriault C: **Interleukin-6 is the main mediator of the interaction between monocytes and platelets in the killing of Schistosoma mansoni**. *European cytokine network* 1990, **1**(1):15-19.
157. Barber DL, Andrade BB, McBerry C, Sereti I, Sher A: **Role of IL-6 in Mycobacterium avium--associated immune reconstitution inflammatory syndrome**. *Journal of immunology* 2014, **192**(2):676-682.
158. Duan HO, Simpson-Haidaris PJ: **Functional analysis of interleukin 6 response elements (IL-6REs) on the human gamma-fibrinogen promoter: binding of hepatic Stat3 correlates negatively**

with transactivation potential of type II IL-6REs. *The Journal of biological chemistry* 2003, **278**(42):41270-41281.
159. Jack DL, Read RC, Tenner AJ, Frosch M, Turner MW, Klein NJ: **Mannose-binding lectin regulates the inflammatory response of human professional phagocytes to Neisseria meningitidis serogroup B**. *The Journal of infectious diseases* 2001, **184**(9):1152-1162.
160. Garred P, Pressler T, Lanng S, Madsen HO, Moser C, Laursen I, Balstrup F, Koch C, Koch C: **Mannose-binding lectin (MBL) therapy in an MBL-deficient patient with severe cystic fibrosis lung disease**. *Pediatric pulmonology* 2002, **33**(3):201-207.
161. Zinyama-Gutsire RB, Chasela C, Madsen HO, Rusakaniko S, Kallestrup P, Christiansen M, Gomo E, Ullum H, Erikstrup C, Munyati S *et al*: **Role of Mannose-Binding Lectin Deficiency in HIV-1 and Schistosoma Infections in a Rural Adult Population in Zimbabwe**. *PloS one* 2015, **10**(4):e0122659.
162. Mombo LE, Lu CY, Ossari S, Bedjabaga I, Sica L, Krishnamoorthy R, Lapoumeroulie C: **Mannose-binding lectin alleles in sub-Saharan Africans and relation with susceptibility to infections**. *Genes and immunity* 2003, **4**(5):362-367.
163. Lawrence RA, Carter T, Bell LV, Else KJ, Summerfield J, Bickle Q: **Altered antibody responses in mannose-binding lectin-A deficient mice do not affect Trichuris muris or Schistosoma mansoni infections**. *Parasite immunology* 2009, **31**(2):104-109.
164. Carter T, Sumiya M, Reilly K, Ahmed R, Sobieszczuk P, Summerfield JA, Lawrence RA: **Mannose-binding lectin A-deficient mice have abrogated antigen-specific IgM responses and increased susceptibility to a nematode infection**. *Journal of immunology* 2007, **178**(8):5116-5123.
165. Meyrowitsch DW, Simonsen PE, Garred P, Dalgaard M, Magesa SM, Alifrangis M: **Association between mannose-binding lectin polymorphisms and Wuchereria bancrofti infection in two communities in North-Eastern Tanzania**. *The American journal of tropical medicine and hygiene* 2010, **82**(1):115-120.
166. Gruden-Movsesijan A, Petrovic M, Sofronic-Milosavljevic L: **Interaction of mannan-binding lectin with Trichinella spiralis glycoproteins, a possible innate immune mechanism**. *Parasite immunology* 2003, **25**(11-12):545-552.
167. Evans-Osses I, Ansa-Addo EA, Inal JM, Ramirez MI: **Involvement of lectin pathway activation in the complement killing of Giardia intestinalis**. *Biochemical and biophysical research communications* 2010, **395**(3):382-386.
168. Asgharzadeh M, Mazloumi A, Kafil HS, Ghazanchaei A: **Mannose-binding lectin gene and promoter polymorphism in visceral leishmaniasis caused by Leishmania infantum**. *Pak J Biol Sci* 2007, **10**(11):1850-1854.
169. de Araujo FJ, Mesquita TG, da Silva LD, de Almeida SA, de SVW, Chrusciak-Talhari A, de OGJA, Talhari S, Ramasawmy R: **Functional variations in MBL2 gene are associated with cutaneous leishmaniasis in the Amazonas state of Brazil**. *Genes and immunity* 2015.
170. Evans-Osses I, Mojoli A, Beltrame MH, da Costa DE, DaRocha WD, Velavan TP, de Messias-Reason I, Ramirez MI: **Differential ability to resist to complement lysis and invade host cells mediated by MBL in R4 and 860 strains of Trypanosoma cruzi**. *FEBS Lett* 2014, **588**(6):956-961.
171. Luz PR, Miyazaki MI, Neto NC, Nisihara RM, Messias-Reason IJ: **High levels of mannose-binding lectin are associated with the risk of severe cardiomyopathy in chronic Chagas Disease**. *Int J Cardiol* 2010, **143**(3):448-450.
172. Weitzel T, Zulantay I, Danquah I, Hamann L, Schumann RR, Apt W, Mockenhaupt FP: **Mannose-binding lectin and Toll-like receptor polymorphisms and Chagas disease in Chile**. *The American journal of tropical medicine and hygiene* 2012, **86**(2):229-232.

173. Klabunde J, Uhlemann AC, Tebo AE, Kimmel J, Schwarz RT, Kremsner PG, Kun JF: **Recognition of plasmodium falciparum proteins by mannan-binding lectin, a component of the human innate immune system**. *Parasitol Res* 2002, **88**(2):113-117.
174. Boldt AB, Luty A, Grobusch MP, Dietz K, Dzeing A, Kombila M, Kremsner PG, Kun JF: **Association of a new mannose-binding lectin variant with severe malaria in Gabonese children**. *Genes and immunity* 2006, **7**(5):393-400.
175. Luty AJ, Kun JF, Kremsner PG: **Mannose-binding lectin plasma levels and gene polymorphisms in Plasmodium falciparum malaria**. *The Journal of infectious diseases* 1998, **178**(4):1221-1224.
176. Holmberg V, Schuster F, Dietz E, Sagarriga Visconti JC, Anemana SD, Bienzle U, Mockenhaupt FP: **Mannose-binding lectin variant associated with severe malaria in young African children**. *Microbes Infect* 2008, **10**(4):342-348.
177. Holmberg V, Onkamo P, Lahtela E, Lahermo P, Bedu-Addo G, Mockenhaupt FP, Meri S: **Mutations of complement lectin pathway genes MBL2 and MASP2 associated with placental malaria**. *Malar J* 2012, **11**:61.
178. Thevenon AD, Leke RG, Suguitan AL, Jr., Zhou JA, Taylor DW: **Genetic polymorphisms of mannose-binding lectin do not influence placental malaria but are associated with preterm deliveries**. *Infection and immunity* 2009, **77**(4):1483-1491.
179. Turner MW: **The role of mannose-binding lectin in health and disease**. *Molecular immunology* 2003, **40**(7):423-429.
180. Torrelles JB, Azad AK, Henning LN, Carlson TK, Schlesinger LS: **Role of C-type lectins in mycobacterial infections**. *Current drug targets* 2008, **9**(2):102-112.
181. Lee SJ, Gonzalez-Aseguinolaza G, Nussenzweig MC: **Disseminated candidiasis and hepatic malarial infection in mannose-binding-lectin-A-deficient mice**. *Mol Cell Biol* 2002, **22**(23):8199-8203.
182. Assaf A, Hoang TV, Faik I, Aebischer T, Kremsner PG, Kun JF, Velavan TP: **Genetic evidence of functional ficolin-2 haplotype as susceptibility factor in cutaneous leishmaniasis**. *PloS one* 2012, **7**(3):e34113.
183. Xiang T, Xiang T, Liu G, Dai WA, Li ZQ, Chen F: **[Study on Ficolin-A against infection of Plasmodium berghei in mouse model]**. *Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi = Chinese journal of parasitology & parasitic diseases* 2014, **32**(1):42-45.
184. Faik I, Oyedeji SI, Idris Z, de Messias-Reason IJ, Lell B, Kremsner PG, Kun JF: **Ficolin-2 levels and genetic polymorphisms of FCN2 in malaria**. *Human immunology* 2011, **72**(1):74-79.
185. Girija UV, Furze CM, Gingras AR, Yoshizaki T, Ohtani K, Marshall JE, Wallis AK, Schwaeble WJ, El-Mezgueldi M, Mitchell DA *et al*: **Molecular basis of sugar recognition by collectin-K1 and the effects of mutations associated with 3MC syndrome**. *BMC biology* 2015, **13**(1):27.
186. Evans-Osses I, de Messias-Reason I, Ramirez MI: **The emerging role of complement lectin pathway in trypanosomatids: molecular bases in activation, genetic deficiencies, susceptibility to infection, and complement system-based therapeutics**. *TheScientificWorldJournal* 2013, **2013**:675898.
187. Boldt AB, Luz PR, Messias-Reason IJ: **MASP2 haplotypes are associated with high risk of cardiomyopathy in chronic Chagas disease**. *Clinical immunology* 2011, **140**(1):63-70.
188. Ribeiro CH, Lynch NJ, Stover CM, Ali YM, Valck C, Noya-Leal F, Schwaeble WJ, Ferreira A: **Deficiency in mannose-binding lectin-associated serine protease-2 does not increase susceptibility to Trypanosoma cruzi infection**. *The American journal of tropical medicine and hygiene* 2015, **92**(2):320-324.
189. La Flamme AC, Scott P, Pearce EJ: **Schistosomiasis delays lesion resolution during Leishmania major infection by impairing parasite killing by macrophages**. *Parasite immunology* 2002, **24**(7):339-345.

190. O'Neal SE, Guimaraes LH, Machado PR, Alcantara L, Morgan DJ, Passos S, Glesby MJ, Carvalho EM: **Influence of helminth infections on the clinical course of and immune response to Leishmania braziliensis cutaneous leishmaniasis**. *The Journal of infectious diseases* 2007, **195**(1):142-148.

ACKNOWLEDGEMENTS

Completion of this doctoral dissertation was possible with the direct and indirect support of several people. I would like to take this opportunity to thank them.

Foremost, I would like to thank Dr. Thirumalaisamy P Velavan and Prof. Peter G Kremsner, for providing me the opportunity to do my PhD thesis at the Institute of Tropical Medicine (ITM), University of Tübingen, Germany. I would like to express my special thanks to my PhD advisor Prof. Peter G Kremsner, Director of the Institute of Tropical Medicine, for his mentorship. I owe my gratitude to Prof. Jürgen Tomiuk for his willingness to be my second supervisor. I would also like to thank my Doctoral committee members, Prof. Dominik Hartl, Prof. Andreas Peschel, Prof. Peter G Kremsner and Prof. Jürgen Tomiuk.

I would like to sincerely thank my direct supervisor, Dr. Thirumalaisamy P Velavan for his guidance. He encouraged me to improve my scientific professionalism, writing skills and independent thinking. I thank Dr. Olusola Ojurongbe, Dr. Thangaraj and Dr. Anshuman Mishra for their support by providing samples. I thank Prof. Michael Kormann for his support. I would like to thank all my colleagues and 'bench mates' of the ITM for their friendship and support. I thank Mr. Timo Grau and Mr. Prabhanjan Gai for their help in genotyping and Mr. Erik Koehne for language check of the thesis. I also thank my Romanian Mother Mrs. Velia Grummes for her help in sequencing.

I thank my friends Mr. Madhu and Mr. Kali for their unconditional support. With all my love I also would like to express my gratitude to my wife Mrs. Renu Justin, my beautiful daughter Ms. Mahilvadhani L Justin, my brother Mr. David, my lovely parents and Dr. Sankar. I also thank my parents in law, and brother in laws for their understanding. I thank all my teachers starting from the primary school till my University for their motivation and encouragement. I also thank my entire village people, as they made me to realize the importance of education.

Last, I dedicate this thesis to Dr. B. R Ambedkar PhD., **"I am because he was"**.

Justin S Antony

Curriculam Vitae

Justin S Antony

PERSONAL DATA

NAME : Justin S Antony
DATE OF BIRTH : May 15th 1985
NATIONALITY : Indian, Passport No. L7687459
MARITUL STATUS : Married, One Daughter
LANGUAGE : English (Full Proficiency), Tamil (Native), German (A1)
ADDRESS : Fichtenweg-9, Apt-215,
72076 Tuebingen
Germany
PHONE: 07071 2982059/017671788935
E-MAIL: justin.s.antony@gmail.com
http://de.linkedin.com/pub/justin-s-antony/17/31b/996/
http://www.researchgate.net/profile/justin_antony2/

ACADEMIC QUALIFICATIONS

2012-2014 **Doctoral Student**, Institute of Tropical Medicine, University of Tübingen.

2006- 2008 **M.Sc., Animal Biotechnology**, Department of Animal Sciences, Bharathidasan University, India **81.0%** First Class with distinction.

2005-2006 **PGDMLT** (PG Diploma in Medical Laboratory Technology)**,** Loyola College, Chennai, **86.0%** First Class with distinction.

2002- 2005 **B.Sc., Zoology,** St.Xaviers College, Palayamkottai, M.S University, India **72.36%** First Class with Merit and Second Rank.

EXPERIENCE

04/2015 TO present **Translational Genomics and Gene therapy**, University of Tübingen
http://www.kormann-lab.de
Research Associate

03/2011 TO 08/2011 **Life Technologies** in Middle East Region (Integrated Gulf Biosystems)
http://www.igbiosystems.com
Field Application Specialist (MCB-Applied Biosystems)

07/2008 TO 01/2011 **Centre for Cellular and Molecular Biology (CCMB)**, Hyderabad, IN
http://www.ccmb.res.in
Senior Research Fellow

SKILLS

Scientific writing
Molecular Biology
Genome Editing (TALENs, ZFNs, CRISPR-Cas9)
Animal models (FELASA-B certified)
Next Generation Sequencing, Sanger Sequencing, Fragment Analysis

GRANTS

Travel grant from GlaxoSmithKline Stiftung for " **HGM-2014**" at Geneva, Switzerland.

PUBLICATIONS

1. **Antony JS**, Ojurongbe O, van Tong H, Ouf EA, Engleitner T, Akindele AA, Sina-Agbaje OR, Adeyeba AO, Kremsner PG, Velavan TP: **Mannose-binding lectin and susceptibility to schistosomiasis**. *J Infect Dis* 2013, 207(11):1675-1683.

2. **Antony JS**, Ojurongbe O, Kremsner PG, Velavan TP: **Lectin complement protein Collectin 11 (CL-K1) and susceptibility to urinary schistosomiasis.** *PLoS Negl Trop Dis* 2015, 9(3):e0003647.

3. **Antony JS**, Ojurongbe O, Meyer CG, Thangaraj K, Mishra A, Kremsner PG, Velavan TP: **Correlation of Interleukin-6 levels and lectins during Schistosoma haematobium infection.** *Cytokine* 2015. doi:10.1016/j.cyto.2015.04.019

4. Mishra A, **Antony JS**, Sundaravadivel P, Tong HV, Meyer CG, Jalli RD, Velavan TP, Thangaraj K: **Association of Ficolin-2 Serum Levels and FCN2 Genetic Variants with Indian Visceral Leishmaniasis.** *PLoS One* 2015, 10(5):e0125940.

5. Ojurongbe O, **Antony JS**, Tong HV, Meyer CG, Akindele AA, Sina-Agbaje OR, Kremsner PG, Velavan TP: **Low MBL-associated serine protease 2 (MASP-2) levels correlate with urogenital schistosomiasis in Nigerian children**. *Trop Med Int Health* **2015**, 20(10):1311-1319.

6. Gehringer C, Kreidenweiss A, Flamen A, **Antony JS**, Grobusch MP, Belard S: **Molecular evidence of Wolbachia endosymbiosis in Mansonella perstans in Gabon, Central Africa.** *J Infect Dis* 2014, 210(10):1633-1638.

Mannose-Binding Lectin and Susceptibility to Schistosomiasis

Justin S. Antony,[1,a] Olusola Ojurongbe,[1,2,a] Hoang van Tong,[1] Eman Abou Ouf,[1] Thomas Engleitner,[1] Akeem A. Akindele,[2] Olawumi R. Sina-Agbaje,[3] Adegboyega O. Adeyeba,[2] Peter G. Kremsner,[1] and Thirumalaisamy P. Velavan[1]

[1]Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany; and [2]Ladoke Akintola University of Technology, Ogbomoso, and [3]Ogun State Hospital, Ijaye Abeokuta, Nigeria

Background. Human ficolin 2 (encoded by *FCN2*) and mannose-binding lectin (encoded by *MBL2*) bind to specific pathogen-associated molecular patterns, activate the complement lectin cascade in a similar manner, and are associated with several infectious diseases. Our recently published study established certain *FCN2* promoter variants and ficolin-2 serum levels as protective factors against schistosomiasis.

Methods. We used the Nigerian cohort from our recently published study, which included 163 *Schistosoma haematobium*–infected individuals and 183 matched healthy subjects, and investigated whether MBL deficiency and *MBL2* polymorphisms are associated with schistosomiasis.

Results. MBL serum levels were significantly higher in controls and were associated with protection ($P < .0001$). The *−550H* minor allele was significantly associated with protection ($P = .03$), and the heterozygous genotypes *−550HL* were observed to confer protection ($P = .03$). The *MBL2*HYPA* haplotype was significantly associated with protection ($P = .03$), with significantly higher serum MBL levels in controls ($P = .00073$). The heterozygous 6-bp deletion in the promoter was observed to be a susceptibility factor in schistosomiasis ($P = .03$).

Conclusions. In agreement with findings from our recently published study, the findings reported here support the observation that MBL is also associated with protection in schistosomiasis.

Keywords. *MBL2*; genotypes; haplotypes; serum level; Schistosomiasis.

Schistosomiasis is one of the neglected tropical diseases targeted for elimination by the World Health Organization and is caused by different species of the trematode genus *Schistosoma*. Of the 207 million estimated cases of schistosomiasis worldwide, 93% (192 million) occur in sub-Saharan Africa, with Nigeria recording the highest number of cases (29 million), followed by the United Republic of Tanzania (19 million) and the Democratic Republic of the Congo and Ghana (15 million each) [1]. Although various species cause schistosomiasis, *Schistosoma haematobium* is the most ubiquitous species in sub-Saharan Africa, contributes to urogenital schistosomiasis, and is associated with severe pathological conditions, such as hematuria, bladder cancer, and hydronephrosis [2–4]. *S. haematobium* infection represents a massive public health problem in Nigeria. It is endemic in many states of the federation, causing immense morbidity, as measured by disability-adjusted life-years, and represents a massive public health problem in Nigeria. The most-affected age group is school-aged children who are constantly in contact with cercariae-infected water [5]. Rapid reinfection [6], a limited supply of praziquantel, and the failure of the host immune system to mount parasite-specific immune responses add to the disease burden [7].

The prevalence of schistosomiasis within communities of endemicity may be influenced by several exposure-related factors, such as local environment and behavior, and by genetic factors related to individual susceptibility [8]. In schistosomiasis, studies have documented that recurrent infections in certain communities are subject to individual variability [9]. Schistosomes are complex multicellular helminthes with different developmental stages in the human host. *S. haematobium* in all developmental stages carries glycoconjugates that

Received 3 October 2012; accepted 18 December 2012; electronically published 28 February 2013.

[a]J. S. A. and O. O. contributed equally to this work.

Correspondence: T. P. Velavan, PhD, Institute of Tropical Medicine, University of Tübingen, Wilhelmstrasse 27, 72074 Tübingen, Germany (velavan@medizin.uni-tuebingen.de).

The Journal of Infectious Diseases 2013;207:1675–83

DOI: 10.1093/infdis/jit081

interact with innate immune recognition elements, such as mannose-binding lectin (MBL) and ficolins. Earlier studies have demonstrated that *Schistosoma* organisms exhibit different glycoproteins on their tegument [10], and carbohydrate moieties remain a potential target for MBL [11]. Moreover, in vitro studies have documented the complement-mediated killing of *Schistosoma mansoni* in all life stages of the parasite [12]. In addition, our previous studies have demonstrated that MBL competently binds to the sugar moieties of *S. mansoni* cercariae and adult worms in vitro and, with MBL-associated serine protease 1 (MASP1) and MASP2, that it is capable of interacting with the *Schistosoma* tegument, thereby activating the complement cascade [13].

MBL is a circulating innate immune recognition protein that is involved in innate immune defense against pathogens [14, 15]. In humans, the MBL encoded by *MBL2* is located on chromosome 10. Three identified single-nucleotide polymorphisms (SNPs) in exon 1 of *MBL2*, at codons 52*(MBL2*D)*, 54*(MBL2*B)*, and 57*(MBL2*C),* interfere with the formation of higher *MBL* oligomers, leading to alterations in functional activity of the protein and their circulating levels [16, 17]. The haplotype of variant alleles is designated as *O*, whereas the haplotype of the common *MBL2* allele is designated as *A* [18]. In addition, 2 strongly linked SNPs in the proximal promoter (*L/H* and *X/Y*), as well as a SNP in the 5′ untranslated region (UTR; P/Q), together are linked to 3 independent nonsynonymous SNPs (ie, B, C, and D) to form "secretor haplotypes," which have been shown to partially account for alterations in complement activation and for decreased circulating levels of MBL [19]. In particular, a base substitution at −221 (G to C; promoter allele *X*) is associated with a lower MBL concentration in serum [11]. Studies have documented that these SNPs contribute to the circulating levels against the 7 common secretor haplotypes (ie, *HYPA*, *HYPD*, *LXPA*, *LYPA*, *LYPB*, *LYQA*, and *LYQC*) [11]. *HYPA*, *LYQA*, and *LYPA* are associated with high expression of MBL, whereas *LXPA*, *HYPD*, *LYPB*, and *LYQC* are associated with low expression of MBL [20]. These secretor haplotypes modulate the MBL concentration in healthy individuals, from levels that are undetectable to levels that are several thousand times greater than the mean level [14, 21]. There is increasing evidence that *MBL2* polymorphisms are associated with infections in pediatric patients, severe acute respiratory syndrome, *Cryptosporidium* infection, human immunodeficiency virus (HIV) infection in children, infections in Polish neonates, visceral leishmaniasis, hepatitis B, malaria and placental malaria, leprosy, and rheumatoid arthritis [22–37].

Despite the documented interaction of *S. mansoni* with the MBL-MASP complex in vitro [38], there is no existing information on the association between functional *MBL2* variants and serum MBL levels and schistosomiasis outcomes. We recently demonstrated that certain *FCN2* promoter variants and ficolin-2 serum levels protected against schistosomiasis. Both ficolins and MBL are similar immune recognition proteins that can bind to carbohydrate sugars, such as N-acetylglucosamine, N-acetylgalactosamine, and glucose, of adult worms and cercariae [39]. Therefore, we hypothesized that MBL plays an analogous role in *S. haematobium* infection. In this study, we investigated the role of *MBL2* polymorphisms and serum MBL levels in *S. haematobium* infection. We examined the possible contribution of functional *MBL2* variants (2 strongly linked SNPs in the proximal promoter, a 6-bp deletion in the promoter, 1 SNP in the 5′ UTR, and 3 SNPs in exon 1) and investigated the relationship of genetic polymorphisms to circulating serum MBL levels in a Nigerian cohort.

PATIENTS AND METHODS

Study Design and Sample Collection

We recruited 346 individuals blindly, irrespective of their infection status, from 2 communities in southwest Nigeria, Ilewo Orile (Abeokuta North) and Ore (Osogbo), in which there was a known history of *S. haematobium* infection. After parasitological and serological testing (for total anti-schistosome immunoglobulin G [IgG] antibodies), the cohort was divided into the following 3 groups: the case group (hereafter, the "SEP group"), the first control group (hereafter, the "SELP group"), and the second control group (hereafter, the "SELN group").

The SEP group was composed of 163 individuals who tested positive for *Schistosoma* eggs. The ratio of males to females was 90 to 73; 99 (61%) were children, of whom 55 (34%) were males, and 44 (27%) were females. The mean age (±SD) was 17.1 ± 12.4 years (range, 4–70 years). Hematuria was detected in 90%, and the mean parasite count was 1595 parasites (range, 20–27 000 parasites).

The SELP group was composed of 119 individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (ELISA). The ratio of males to females was 58 to 61; 23 (19%) were children, of whom 10 (8%) were males, and 13 (11%) were females. The mean age (±SD) was 33.6 ± 18.8 years (range, 4–75 years). Hematuria was detected in 2.4%.

The SELN group was composed of 64 individuals who tested negative for *Schistosoma* antigens by ELISA and negative for *Schistosoma* eggs. The ratio of males to females was 40 to 24; 31 (48%) were children, of whom 23 (36%) were males, and 8 (13%) were females. The mean age (±SD) was 20.2 ± 17.2 years (range, 4–71 years). Hematuria was not detected in any subject.

All subjects were of Yoruba ethnicity. Additional parasitological data, serological data, and characteristics of this cohort are reported elsewhere [39].

About 10 mL of urine from all participants was collected into a sterile, labeled container and examined for the presence of *S. haematobium* eggs, and about 5 mL of blood was collected for serological assays and DNA extraction. Details about the

study were provided in the local language to potential participants, and informed consent was obtained before recruitment into the study; for children, consent was obtained from parents and/or guardians. Ethics approval was obtained from the Ethical Committee of Ministry of Health, Abeokuta Ogun State, Nigeria.

Serological Assays

Serological assays were performed using a modified ELISA protocol to detect total anti-schistosome IgG antibodies, as previously described [40]. Serum levels of MBL were measured in patients and healthy control subjects, using an ELISA kit for human MBL (Hycult Biotech, Uden, the Netherlands).

MBL2 Genotyping

Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The *MBL2* promoter polymorphisms at −550G > C, −221G > C, and the 5′ UTR +4C > T and the exon 1 polymorphisms at codons 52C > T, 54G > A, and 57G > A were amplified by polymerase chain reaction (PCR), using primer pairs spanning from the promoter region through exon 1. The primers used were as follows: promoter, forward: 5′-GCCAGAAAGTAGAGAGGTATTTAG CAC-3′; internal primer exon 1, forward: 5′-CAGGTGTCT AGGCACAGATGAACC-3′; and exon 1, reverse: 5′-CCAA CACGTACCTGGTTCCC-3′. In brief, 10 ng of genomic DNA was amplified in a 20-μL volume of reaction mixture containing 1× PCR buffer (20 mM Tris-HCl [pH 8.4], 50 mM KCl, and 1.5 mM of $MgCl_2$), 0.125 mM of dNTPs, 0.25 mM of each primer, and 1 U Taq DNA polymerase (Qiagen, Hilden, Germany) on a PTC-200 Thermal cycler (MJ Research, United States). Thermal cycling parameters for amplification of both exon 1 and promoter regions were 94°C for 5 minutes (initial denaturation); 35 cycles of 30 seconds at 94°C (for denaturation), 30 seconds at 68°C (for annealing), 90 seconds at 72°C (for extension); and 120 second at 72°C (for final extension). PCR products were cleaned up by use of Exo-SAP-IT (USB, Affymetrix, United States), and 1 μL of the purified product was directly used as a template for sequencing, using the BigDye terminator v. 1.1 cycle sequencing kit (Applied Biosystems, United States) on an ABI 3130XL DNA sequencer, according to the manufacturers' instructions. Polymorphisms were identified by assembling the sequences with respective reference sequences (NG_008196.1), using CodonCode Aligner, version 4.0 (available at: http://www.codoncode.com/), and were reconfirmed visually from their respective electropherograms.

Statistical Analysis

Data were analyzed using Intercooled Stata, version 9.1, and the level of significance was set as a P value of < .05. A Kruskal-Wallis test was used to analyze the correlation of serum MBL levels to *MBL2* variants. A 2-tailed Fischer exact test was performed to determine the differences in allele frequencies and genotype distributions. The Benjamini-Hochberg procedure was used for correction of P values involving multiple comparisons. Genotype or haplotype frequencies were analyzed by simple gene counting and the expectation-maximum algorithm, and the statistical significance of deviations from Hardy-Weinberg equilibrium was tested using the random-permutation procedure, as implemented in Arlequin software, version 3.5.1.2 (available at: http://cmpg.unibe.ch/software/arlequin3/). Linkage disequilibrium analysis was performed using Haploview, version 3.2.

RESULTS

MBL2 Gene Polymorphisms and *S. haematobium* Infection

Both genotype and allele frequencies for all analyzed functional *MBL2* SNPs in each respective group were in Hardy-Weinberg equilibrium. The distribution of *MBL2* genotypes and allele(s) in the cohort are shown in Table 1. Linkage disequilibrium patterns of studied *MBL2* variants, along with the observed 6-bp deletion in the SEP group, the SELP group, the SELN group, and the SELP and SELN groups combined are shown in Figure 1. The 6-bp deletion was in linkage disequilibrium with the promoter variant +4C/T (P/Q) in all studied subgroups.

Significant differences were observed either in genotypes or allelic distributions between the SEP group and both the SELN group alone and the SELP and SELN groups combined for the −550G/C variant. The homozygous variant *LL* (−550CC) genotype was observed more frequently in the SEP group, compared with the SELN group alone (odds ratio [OR], 2.7; 95% CI, 1.08–6.65; $P = .02$) and the SELP and SELN groups combined (OR, 2.1; 95% CI, 1.06–4.59; $P = .03$), suggesting that it is associated with an increased risk for *S. haematobium* infection (Table 1). The heterozygous genotype *HL* (−550GC) was observed less frequently in the SEP group, compared with the SELN group alone (OR, 0.34; 95% CI, .13–.86; $P = .01$) and the SELP and SELN groups combined (OR, 0.44; 95% CI, .20–.91; $P = .03$), suggesting that it might be protective against *S. haematobium* infection. The minor allele −550*H* was observed more frequently in the SELN group alone (OR, 0.42; 95% CI, .18–1.0; $P = .048$) and the SELP and SELN groups combined (OR, 0.5; 95% CI, .24–.98; $P = .03$), compared with the SEP group (Table 1). In exon 1, heterozygous genotype *PQ* (+4CT) was observed more frequently in the SEP group, compared with the SELN group (OR, 2.27; 95% CI, 1.2–4.31; $P = .02$). The homozygous genotype *PP* (+4CC) was observed less frequently in the SEP group, compared with the SELN group (OR, 0.4; 95% CI, .2–.9; $P = .02$). No significant differences in any other genotype distribution were observed when the SEP group was compared with the SELP group (data not shown). The heterozygous 6-bp deletion in the promoter region (−338 to −332) was more

Table 1. Distribution of *MBL2* Genotypes and Alleles Among Cases and Controls

SNP Position	Genotype	Subjects, No. (%), by Study Group(s)				SEP vs SELN		SEP vs (SELP + SELN)	
		SEP (n = 163)	SELP (n = 119)	SELN (n = 64)	SELP + SELN (n = 183)	OR (95% CI)	P^a	OR (95% CI)	P^a
rs11003125 (–550G/C)									
CC	LL	149 (91.4)	101 (84.9)	51 (79.7)	152 (83.1)	**2.7 (1.08–6.65)**	**.02**	**2.1 (1.06–4.59)**	**.03**
GC	HL	13 (8)	17 (14.3)	13 (20.3)	30 (16.4)	**0.34 (.13–.86)**	**.01**	**0.44 (.20–.91)**	**.03**
GG	HH	1 (0.6)	1 (0.8)	0	1 (0.5)	. . .	NS	. . .	
C	L	311 (95.4)	219 (92)	115 (89.8)	334 (91.3)	. . .		. . .	
G	H	15 (4.6)	19 (8)	13 (10.2)	32 (8.7)	**0.42 (.18–1.0)**	**.048**	**0.50 (.24–.98)**	**.03**
rs7096006 (–221G/C)									
GG	YY	126 (77.3)	98 (82.3)	46 (71.9)	144 (78.7)	. . .		. . .	
GC	YX	33 (20.2)	19 (16)	15 (23.4)	34 (18.6)	. . .		. . .	
CC	XX	4 (2.5)	2 (1.7)	3 (4.7)	5 (2.7)	. . .	NS	. . .	
G	Y	285 (87.4)	215 (90.3)	107 (83.6)	322 (88)	. . .		. . .	
C	X	41 (12.6)	23 (9.7)	21 (16.4)	44 (12)	. . .		. . .	
rs7095891 (+4C/T)									
CC	PP	23 (14.1)	15 (12.6)	18 (28.1)	33 (18.1)	**0.4 (.2–.9)**	**.02**	. . .	
CT	PQ	94 (57.7)	61 (51.3)	24 (37.5)	85 (46.4)	**2.27 (1.2–4.31)**	**.02**	. . .	
TT	QQ	46 (28.2)	43 (36.1)	22 (34.4)	65 (35.5)	. . .	NS	. . .	
C	P	140 (42.9)	91 (38.2)	60 (46.9)	151 (41.3)	. . .		. . .	
T	Q	186 (57.1)	147 (61.8)	68 (53.1)	215 (58.7)	. . .	NS	. . .	
*MBL2** exon 1 (codon 57)									
GG	AA	81 (49.7)	56 (46.2)	35 (54.7)	91 (49.7)	. . .		. . .	
GA	AO	71 (43.6)	50 (42.9)	27 (42.2)	77 (42.0)	. . .		. . .	
AA	OO	11 (6.7)	13 (10.9)	2 (3.1)	15 (8.3)	. . .	NS	. . .	
G	A	233 (71.5)	162 (68.1)	97 (75.8)	259 (70.8)	. . .		. . .	
A	O	93 (28.5)	76 (31.9)	31 (24.2)	107 (29.2)	. . .		. . .	
6-bp deletion (–338 to –332)									
	wt/wt	25 (15.3)	17 (14.3)	17 (26.6)	34 (18.6)	. . .	NS	. . .	
	wt/del	92 (56.5)	56 (47)	24 (37.5)	80 (43.7)	**2.15 (1.1–4.09)**	**.03**	. . .	
	del/del	46 (28.2)	46 (38.7)	23 (35.9)	69 (37.7)	. . .	NS	. . .	
	wt	142 (43.5)	90 (37.8)	58 (45.5)	148 (40.4)	. . .	NS	. . .	
	del	184 (56.5)	148 (62.2)	70 (54.5)	218 (59.6)	. . .		. . .	
Promoter (–221) + exon 1									
	YA/YA	55 (33.7)	43 (36.1)	19 (29.7)	62 (33.9)	. . .		. . .	
	YA/YO	60 (36.8)	42 (35.4)	25 (39.1)	67 (36.6)	. . .		. . .	
	YO/YO	11 (6.7)	13 (10.9)	2 (3.1)	15 (8.2)	. . .		. . .	
	XA/YA	22 (13.5)	11 (9.2)	13 (20.3)	24 (13.1)	. . .	NS	. . .	
	XA/YO	11 (6.7)	8 (6.7)	2 (3.1)	10 (5.5)	. . .		. . .	
	XA/XA	4 (2.4)	2 (1.7)	3 (4.7)	5 (2.7)	. . .		. . .	

Percentages may not sum to 100%, because of rounding errors. Study groups were as follows: the SEP group (cases) was composed of individuals who tested positive for *Schistosoma* eggs, the SELP group (controls) was composed of individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (ELISA), and the SELN group (controls) was composed of individuals who tested negative for *Schistosoma* antigens by ELISA and negative for *Schistosoma* eggs.

Abbreviations: CI, confidence interval; NS, not significant; OR, odds ratio.

[a] Adjusted by the Benjamini-Hochberg procedure.

frequent in the SEP group, compared with the SELN group (OR, 2.15; 95% CI, 1.1–4.09; $P = .01$).

No significant differences were observed for other genotype comparisons between the cases and both control groups. We only observed variants at codon 57*(MBL2*C)*, and no variants were observed at codons 52*(MBL2*D)* and 54*(MBL2*B)* in all the studied subgroups. When the combined genotypes for variant −221G/C and exon 1 (at codons 52*[MBL2*D]*, 54*[MBL2*B]*, and 57*[MBL2*C]*) were investigated for their influence in serum MBL levels (high MBL producers [*YA/YA*],

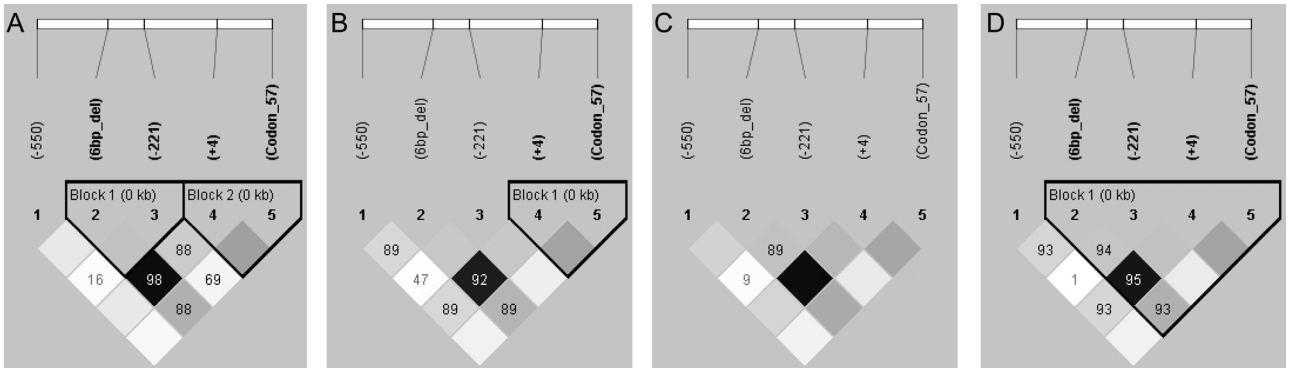

Figure 1. Linkage disequilibrium pattern of *MBL2* variants in individuals who tested positive for *Schistosoma* eggs (the SEP group [cases]; *A*), individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (ELISA; the SELP group [controls]; *B*), individuals who tested negative for *Schistosoma* antigens by ELISA and negative for *Schistosoma* eggs (the SELN group [controls]; *C*), and the SELP and SELN groups combined (*D*). Empty squares indicate a high degree of linkage disequilibrium (D′ = 1). Numbers indicate the D′ value expressed as a percentile. The dense shading indicates the r^2 value. The haplotype block is outlined by a solid line.

intermediate producers [*YA/YO*, *XA/XA*, and *XA/YA*], and low producers [*YO/YO*, *XA/YO*, and *XA/XO*]), we observed no significant differences in comparisons between cases and controls (Table 1).

The reconstructed *MBL2* haplotypes and the observed distribution in the Nigerian cohort are summarized in Table 2. We observed only 5 secretor haplotypes in our study. The *MBL2* haplotypes were divided into those associated with high expression (*LYPA* + *LYQA* + *HYPA*) and those associated with low expression (*LYQC* + *LXPA*) of MBL. No significantly different distributions were observed (Table 2). The reconstructed haplotypes were marginally significantly different in comparisons between the SEP group and the SELN group alone (OR, 0.4; 95% CI, .18–1; $P = .048$) and the SELP and SELN groups combined (OR, 0.5; 95% CI, .25–98; $P = .03$), providing genetic clues about a possible factor of S. *haematobium* susceptibility (Table 2). No significant differences in haplotype distribution were observed between the SEP group and the SELP group (data not shown).

MBL Serum Levels and *S. haematobium* Infection

The serum MBL levels varied significantly between the SEP and SELP groups ($P < .0001$; Figure 2*A*). Also, the serum MBL levels were significantly higher in the SELP and SELN groups combined, compared with the SEP group ($P < .0001$; Figure 2*A*). The median MBL levels detected within groups were 27.6 ng/mL

Table 2. Distribution of *MBL2* Haplotypes in Cases and Controls

	Haplotypes, No. (%), by Study Group(s)				SEP vs SELN		SEP vs (SELP + SELN)	
Haplotype	SEP (n = 326)	SELP (n = 238)	SELN (n = 128)	SELP + SELN (n = 366)	OR (95% CI)	P^a	OR (95% CI)	P^a
MBL2 LXPA*	41 (12.6)	23 (9.7)	21 (16.4)	44 (12)	. . .	NS	. . .	NS
MBL2 LYQC*	93 (28.5)	77 (32.3)	31 (24.2)	108 (29.5)	. . .	NS	. . .	NS
MBL2 LYQA*	93 (28.5)	70 (29.4)	37 (28.9)	107 (29.3)	. . .	NS	. . .	NS
MBL2 HYPA*	15 (4.6)	19 (8)	13 (10.2)	32 (8.7)	**0.4 (.18–1)**	**.048**	**0.5 (.25–.98)**	**.03**
MBL2 LYPA*	84 (25.8)	49 (20.6)	26 (20.3)	75 (20.5)	. . .	NS	. . .	NS
Low expression of *MBL2**								
LYQC + LXPA	134 (41.1)	100 (42)	52 (40.6)	152 (41.5)	. . .	NS	. . .	NS
High expression of *MBL2**								
HYPA + LYQA + LYPA	192 (58.9)	138 (58)	76 (59.4)	214 (58.5)	. . .	NS	. . .	NS

Percentages may not sum to 100%, because of rounding errors. Study groups were as follows: the SEP group (cases) was composed of individuals who tested positive for *Schistosoma* eggs, the SELP group (controls) was composed of individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (ELISA), and the SELN group (controls) was composed of individuals who tested negative for *Schistosoma* antigens by ELISA and negative for *Schistosoma* eggs.

Abbreviations: CI, confidence interval; NS, not significant; OR, odds ratio.

[a] Adjusted by the Benjamini-Hochberg procedure.

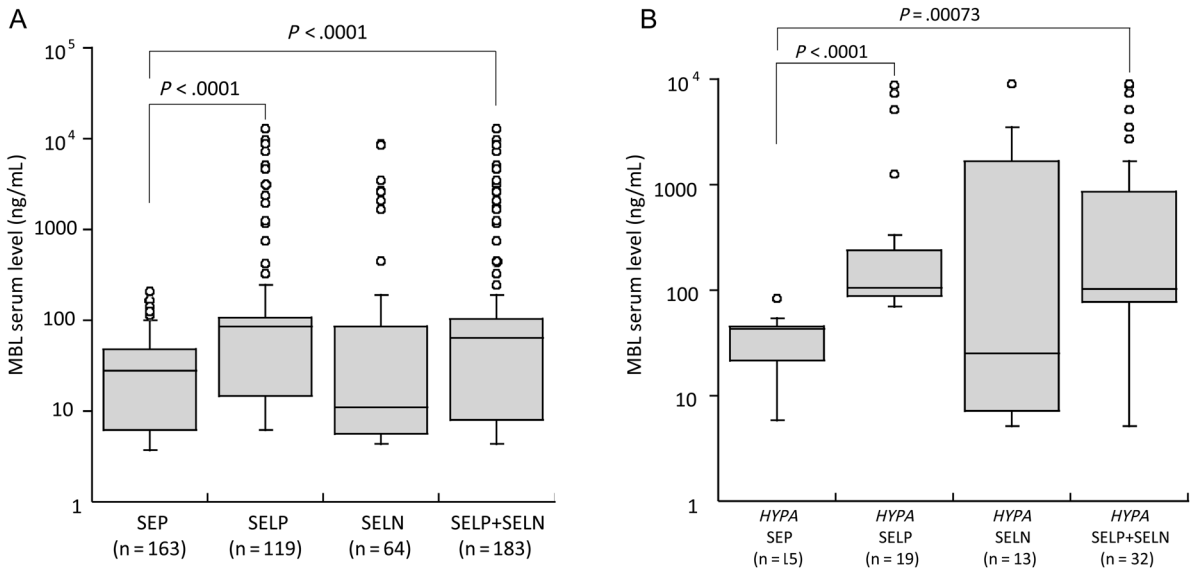

Figure 2. *A*, Mannose-binding lectin (MBL) serum levels in individuals who tested positive for *Schistosoma* eggs (the SEP group [cases]), individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (ELISA; the SELP group [controls]), individuals who tested negative for *Schistosoma* antigens by ELISA and negative for *Schistosoma* eggs (the SELN group [controls]), and the SELP and SELN groups combined. *B*, MBL serum levels in individuals with *HYPA* haplotype in cases and controls.

(range, 3.3–209 ng/mL) for the SEP group, 84.06 ng/mL (range, 6.13–13 122 ng/mL) for the SELP group, and 11.24 ng/mL (range, 4.38–8936 ng/mL) for the SELN group. Similar results from our recently published study demonstrated that higher serum ficolin-2 levels shield against schistosomiasis [39]. The serum MBL level of the reconstructed *MBL2*HYPA* haplotype for the SEP group was significantly lower than that for the SELP group alone ($P < .0001$) and the SELP and SELN groups combined ($P < .0001$; Figure 2*B*). We observed a significantly different distribution of serum MBL levels both in the SEP group and the SELP group among the investigated genotypes that were associated with differential serum MBL levels (Figure 3). In both observed distributions, the genotype *YA/YA* (high MBL producers) had an increased MBL levels, compared with the other genotypes in the studied population. No significant correlations were observed with the egg counts and serum levels (data not shown).

DISCUSSION

To the best of our knowledge, this is the first study that investigated the role of *MBL2* functional gene polymorphisms and MBL serum levels in schistosomiasis susceptibility. In our recent study, we demonstrated that *FCN2* polymorphisms and ficolin-2 levels influence the outcome of schistosomiasis [39]. Similar to ficolins, MBL is an innate immune recognition protein that plays a vital role during the early phase of infection and pathogen recognition. Additionally, our studies have documented the involvement of MBL-activated complement in the response to *Schistosoma* antigens in vitro [13]. Therefore, in this study, we hypothesized that the sugar-rich surface of *Schistosoma* organisms is a good target for MBL and that single-locus substitutions in *MBL2* resulting in variation in MBL production would eventually affect the disease outcome. In this study, we investigated the possible association of MBL serum levels and *MBL2* variants in a case-control study, using 2 independent control groups. The classification of the control group is indispensable in *Schistosoma*-endemic areas, as it is difficult to differentiate individuals with and without a current infection.

MBL deficiency is associated with many diseases and protection against intracellular infections, such as tuberculosis, leprosy, and leishmaniasis [27, 32, 37, 41]. In our study, we observed that promoter genotypes (*−550H/L*) influence predisposition to schistosomiasis. The heterozygous genotype (*−550HL*) conferred protection against infection, whereas the homozygous genotype (*−550LL*) contributed to increased susceptibility. The results remained consistent when egg-positive patients (the SEP group) were compared to individuals in the SELN group, with or without individuals in the SELP group. The *−550H* minor allele in the studied population decreased the risk of *Schistosoma* infection. Our results imply that individuals who are heterozygous at promoter position *−550H/L* are less susceptible to infection. As observed in our study, earlier studies have documented the influence of *−550H/L* variants on the risk of HIV infection and AIDS progression in Italian children [34] and on prosthetic joint infection in Czech

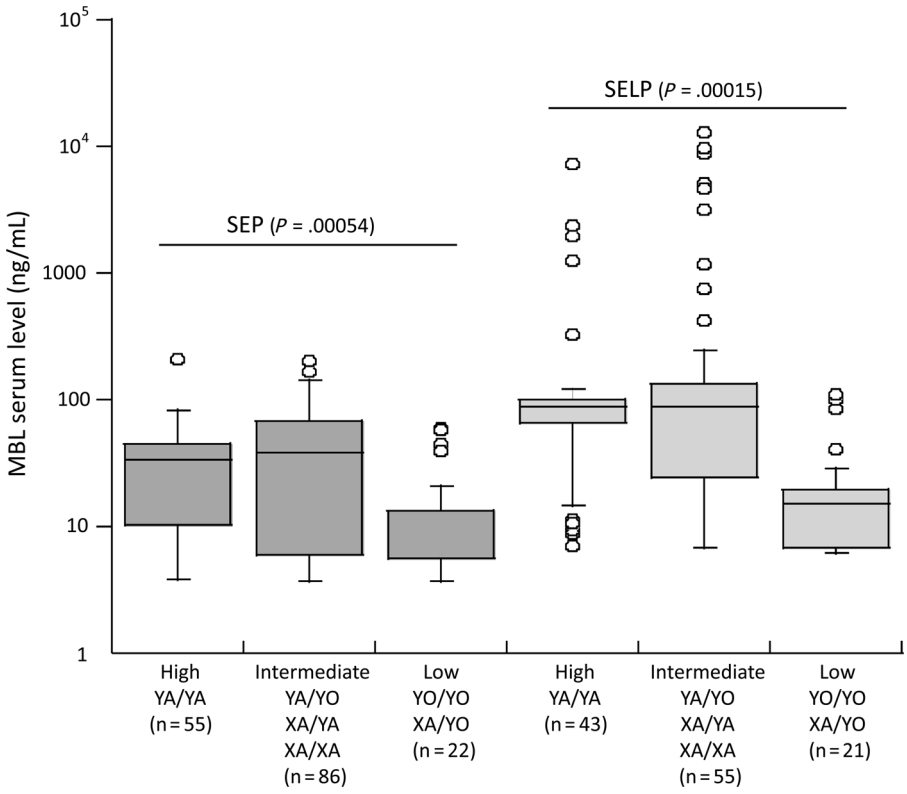

Figure 3. Mannose-binding lectin (MBL) serum levels according to combined genotypes of the −221 promoter region and exon 1 of *MBL2* in individuals who tested positive for *Schistosoma* eggs (the SEP group) and individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (the SELP group). Genotypes were divided into high MBL-producing genotypes (*YA/YA*), intermediate MBL-producing genotypes (*YA/YO, XA/YA,* and *XA/XA*), and low MBL-producing genotypes (*YO/YO* and *XA/YO*).

patients [42]. However, another study, which investigated the association of *MBL2* variants with Bechet disease in a South Korean population, demonstrated that the patients had lower frequencies of the homozygous genotype (*−550LL*) [43]. This variation emphasizes that the clinical significance of the *MBL2* variants may likely depend on the population and the disease context. We also observed that a noncoding SNP located +4 bp from the transcription start site (+4P/Q) was also observed to influence the predisposition to schistosomiasis, as determined by comparison of the SEP group to the SELN group. The individuals with major allele homozygous genotypes (+4PP) were less susceptible to infection than those in the SELN group, whereas the individuals with heterozygous genotypes (+4PQ) had a higher risk of infection than those in the SELN group. However, we did not observe significant associations with pooled values for the control groups.

In our studied population, we only observed the *MBL2*C* genotype (codon 57). No significant associations were observed in the distribution of genotypes (*MBL2*C* independently and with the combined promoter-220 + exon 1) that influences the serum MBL levels. This observed *MBL2*C* genotype was documented to be the most frequent African *MBL2* variant [31], and studies have reported that the *MBL2*C* variant is associated with placental malaria and malaria in African children [31, 44]. A heterozygous 6-bp deletion (wt/del) in the promoter region was observed to confer increased risk to schistosomiasis in comparisons between the SEP group and the SELN group. These results are in accordance with those of another study, which demonstrated that the 6-bp deletion at position −328 was correlated with HIV type 1 infection [34]. The reconstruction of haplotypes is important to revealing how different combinations of functional polymorphic alleles can interact to modulate their individual effects. Moreover, many studies have shown the effect of *MBL2* haplotypes in disease association studies [32, 35, 43]. Stratification of our cohort on the basis of *MBL2* haplotype showed that the *MBL2*HYPA* haplotype was significantly lower in the case group than in controls, elucidating the fact that individuals with these haplotypes had a lower risk of schistosomiasis. Similar results on the influence of *MBL2*HYPA* haplotypes has been demonstrated in Bechet disease, in pediatric patients with common infectious diseases, and in patients with colon cancer [23, 43, 45].

Investigation of the MBL serum level further confirms the important role of MBL in schistosomiasis. MBL serum levels

varied significantly between cases and controls. A significant difference was found between the SEP group and both the SELP group alone and the SELP and SELN groups combined. MBL serum levels were significantly higher in the control groups, compared with the case group, showing that high levels of MBL may have a protective role in reducing the susceptibility to *S. haematobium* infection and adding further support the view that MBL plays a role in first-line defense against this pathogen. The *MBL2*HYPA* haplotypes that contributed to a decreased risk of schistosomiasis were also correlated significantly to MBL serum levels. The control groups (the SELP group alone and the SELP and SELN groups combined) had significantly higher MBL serum levels, compared with the case group. *MBL2* polymorphisms related to higher MBL levels in serum were associated with decreased susceptibility to respiratory tract infection in children [46] and to SARS coronavirus infection, compared with controls [22]. The role of MBL as a first line of defense in infectious and noninfectious diseases has been documented in many clinical settings. Comparable results from our study were reported in individuals infected with the intestinal protozoan parasite *Cryptosporidium*, in which *MBL2* polymorphisms and haplotypes that were associated with deficient serum MBL levels increased the susceptibility to infection [30]. On the contrary, *MBL2* polymorphisms were also reported to play a dual role in leprosy [32]: the wild-type haplotype was shown to increase susceptibility to the disease per se, and defective haplotypes were shown to confer protection against lepromatous and borderline leprosy. Therefore, depending on the type of disease, MBL deficiency may increase susceptibility to intestinal parasite infections, as in cryptosporidium and schistosomiasis, or may be protective against intracellular pathogens, such as *Mycobacterium leprae* [32]. In our study, a normal MBL level was found to decrease the susceptibility to *S. haematobium* infection.

In our earlier study, we observed that ficolin-2 gene polymorphisms and ficolin-2 serum level play an important role in the susceptibility to *S. haematobium* infection [39]. Both MBL and L-ficolin have similar structural templates comprising collagen-like and ligand binding domains that binds specifically to pathogen-associated molecular patterns on the pathogen surface. In association with the MBL-associated serine proteases (MASP1 and MASP2), MBL and L-ficolin initiate the complement lectin cascade, leading to clearance of pathogens by opsonization [47, 48]. A previous study showed that the surfaces of both *S. mansoni* cercariae and adult worms were recognized specifically by the MBL-MASP complex, suggesting that an interaction between MBL and the parasite results in activation of complement in vitro [13]. Lectin-binding studies of *Schistosoma* organisms have revealed many different glycoproteins on the surface of the parasite, regardless of life cycle stage and sex [38, 49], and it was shown MBL interacts with these sugar residues on the parasite surface, triggering the host immune response [13, 50]. We clearly observed in this study that higher MBL levels and ficolin-2 levels [39] were predictive of protection from *S. haematobium* infection.

The study shows that there is still a high prevalence of *S. haematobium* infection in Nigeria, with children bearing the highest burden. Overall, we demonstrated that *MBL2* variants and serum MBL levels are associated with protection against *S. haematobium* infection. This study, in line with our previous study of ficolins, may provide vital insights about the possible role of MBL, a vital innate immune component of the complement system that regulates *Schistosoma* infection and may lay the foundation for a testable immunological intervention to control and contain the disease in human populations.

Notes

Acknowledgments. We thank Abass Busari of State Hospital Ijaye, Abeokuta; Sanusi Tawakalitu, Olaolu Abdulazeez, and Adedokun Samuel, of Ladoke Akintola University of Technology, Osogbo, for the technical support; the staff of the Schistosomiasis Control Unit, Ministry of Health, Abeokuta, and the community members, for their participation in the study; Silvelia Grummes, for technical help; and Timo Grau, for help with sequencing.

Financial support. This work was funded by an institutional internal grant, the Alexander von Humboldt Foundation (to O. O.), the Egyptian Ministry of Higher Education (to E. A. O.), and the Vietnamese government (scholarship to H. v. T.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis **2006**; 6:411–25.
2. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. World Health Organ Tech Rep Ser **2002**; 912:1–57.
3. Akinwale OP, Oliveira GC, Ajayi MB, Akande DO, Oyebadejo S, Okereke KC. Squamous cell abnormalities in exfoliated cells from the urine of *Schistosoma haematobium*-infected adults in a rural fishing community in Nigeria. World Health Popul **2008**; 10:18–22.
4. van der Werf MJ, de Vlas SJ, Brooker S, et al. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. Acta Trop **2003**; 86:125–39.
5. Mafe MA, Appelt B, Adewale B, et al. Effectiveness of different approaches to mass delivery of praziquantel among school-aged children in rural communities in Nigeria. Acta Trop **2005**; 93:181–90.
6. Uneke CJ. Soil transmitted helminth infections and schistosomiasis in school age children in sub-Saharan Africa: efficacy of chemotherapeutic intervention since World Health Assembly Resolution 2001. Tanzan J Health Res **2010**; 12:86–99.
7. Yazdanbakhsh M, Sacks DL. Why does immunity to parasites take so long to develop? Nat Rev Immunol **2010**; 10:80–1.
8. Pinot de MA, Fulford AJ, Kabatereine NB, Ouma JH, Booth M, Dunne DW. Analysis of complex patterns of human exposure and immunity to *Schistosoma mansoni*: the influence of age, sex, ethnicity and IgE. PLoS Negl Trop Dis **2010**; 4:1–10.
9. Dessein AJ, Couissinier P, Demeure C, et al. Environmental, genetic and immunological factors in human resistance to *Schistosoma mansoni*. Immunol Invest **1992**; 21:423–53.

10. Hayunga EG, Sumner MP. Expression of lectin-binding surface glycoproteins during the development of *Schistosoma mansoni* schistosomula. J Parasitol **1986**; 72:913–20.
11. Dommett RM, Klein N, Turner MW. Mannose-binding lectin in innate immunity: past, present and future. Tissue Antigens **2006**; 68:193–209.
12. Rasmussen KR, Kemp WM. *Schistosoma mansoni*: interactions of adult parasites with the complement system. Parasite Immunol **1987**; 9:235–48.
13. Klabunde J, Berger J, Jensenius JC, et al. *Schistosoma mansoni*: adhesion of mannan-binding lectin to surface glycoproteins of cercariae and adult worms. Exp Parasitol **2000**; 95:231–9.
14. Ip WK, Takahashi K, Ezekowitz RA, Stuart LM. Mannose-binding lectin and innate immunity. Immunol Rev **2009**; 230:9–21.
15. Kilpatrick DC. Introduction to mannan-binding lectin. Biochem Soc Trans **2003**; 31(Pt 4):745–7.
16. Garred P, Larsen F, Madsen HO, Koch C. Mannose-binding lectin deficiency—revisited. Mol Immunol **2003**; 40:73–84.
17. Garred P, Larsen F, Seyfarth J, Fujita R, Madsen HO. Mannose-binding lectin and its genetic variants. Genes Immun **2006**; 7:85–94.
18. Velavan TP, Boldt AB, Tomiuk J, et al. Variant alleles of the mannose binding lectin 2 gene (MBL2) confer heterozygote advantage within Crohn's families. Scand J Gastroenterol **2010**; 45:1129–30.
19. Madsen HO, Garred P, Thiel S, et al. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol **1995**; 155:3013–20.
20. Madsen HO, Satz ML, Hogh B, Svejgaard A, Garred P. Different molecular events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America. J Immunol **1998**; 161:3169–75.
21. Ezekowitz RA, Day LE, Herman GA. A human mannose-binding protein is an acute-phase reactant that shares sequence homology with other vertebrate lectins. J Exp Med **1988**; 167:1034–46.
22. Zhang H, Zhou G, Zhi L, et al. Association between mannose-binding lectin gene polymorphisms and susceptibility to severe acute respiratory syndrome coronavirus infection. J Infect Dis **2005**; 192:1355–61.
23. Tao R, Hua CZ, Hu YZ, Shang SQ. Genetic polymorphisms and serum levels of mannose-binding lectin in Chinese pediatric patients with common infectious diseases. Int J Infect Dis **2012**; 16:e403–7.
24. Swierzko AS, Atkinson AP, Cedzynski M, et al. Two factors of the lectin pathway of complement, l-ficolin and mannan-binding lectin, and their associations with prematurity, low birthweight and infections in a large cohort of Polish neonates. Mol Immunol **2009**; 46:551–8.
25. Summerfield JA, Ryder S, Sumiya M, et al. Mannose binding protein gene mutations associated with unusual and severe infections in adults. Lancet **1995**; 345:886–9.
26. Song LH, Binh VQ, Duy DN, et al. Mannose-binding lectin gene polymorphisms and hepatitis B virus infection in Vietnamese patients. Mutat Res **2003**; 522:119–25.
27. Santos IK, Costa CH, Krieger H, et al. Mannan-binding lectin enhances susceptibility to visceral leishmaniasis. Infect Immun **2001**; 69:5212–5.
28. Martiny FL, Veit TD, Brenol CV, et al. Mannose-binding lectin gene polymorphisms in Brazilian patients with rheumatoid arthritis. J Rheumatol **2012**; 39:6–9.
29. Luty AJ, Kun JF, Kremsner PG. Mannose-binding lectin plasma levels and gene polymorphisms in *Plasmodium falciparum* malaria. J Infect Dis **1998**; 178:1221–4.
30. Kelly P, Jack DL, Naeem A, et al. Mannose-binding lectin is a component of innate mucosal defense against *Cryptosporidium parvum* in AIDS. Gastroenterology **2000**; 119:1236–42.
31. Holmberg V, Onkamo P, Lahtela E, et al. Mutations of complement lectin pathway genes MBL2 and MASP2 associated with placental malaria. Malar J **2012**; 11:61.
32. de Messias-Reason IJ, Boldt AB, Moraes Braga AC, et al. The association between mannan-binding lectin gene polymorphism and clinical leprosy: new insight into an old paradigm. J Infect Dis **2007**; 196:1379–85.
33. Cedzynski M, Swierzko AS, Kilpatrick DC. Factors of the lectin pathway of complement activation and their clinical associations in neonates. J Biomed Biotechnol **2012**; 2012:363246.
34. Boniotto M, Crovella S, Pirulli D, et al. Polymorphisms in the MBL2 promoter correlated with risk of HIV-1 vertical transmission and AIDS progression. Genes Immun **2000**; 1:346–8.
35. Boldt AB, Messias-Reason IJ, Lell B, et al. Haplotype specific-sequencing reveals MBL2 association with asymptomatic *Plasmodium falciparum* infection. Malar J **2009**; 8:97.
36. Boldt AB, Luty A, Grobusch MP, et al. Association of a new mannose-binding lectin variant with severe malaria in Gabonese children. Genes Immun **2006**; 7:393–400.
37. Alonso DP, Ferreira AF, Ribolla PE, et al. Genotypes of the mannan-binding lectin gene and susceptibility to visceral leishmaniasis and clinical complications. J Infect Dis **2007**; 195:1212–7.
38. Schmidt J. Glycans with N-acetyllactosamine type 2-like residues covering adult Schistosoma mansoni, and glycomimesis as a putative mechanism of immune evasion. Parasitology **1995**; 111(Pt 3):325–36.
39. Ouf EA, Ojurongbe O, Akindele AA, et al. Ficolin-2 levels and FCN2 genetic polymorphisms as a susceptibility factor in schistosomiasis. J Infect Dis **2012**; 206:562–70.
40. Knobloch J, Delgado E. Immunodiagnosis of cysticercosis: standardization of ELISA and its application to field conditions. Trop Med Parasitol **1985**; 36:157–9.
41. Cosar H, Ozkinay F, Onay H, et al. Low levels of mannose-binding lectin confers protection against tuberculosis in Turkish children. Eur J Clin Microbiol Infect Dis **2008**; 27:1165–9.
42. Navratilova Z, Gallo J, Mrazek F, Lostak J, Petrek M. MBL2 gene variation affecting serum MBL is associated with prosthetic joint infection in Czech patients after total joint arthroplasty. Tissue Antigens **2012**; 80:444–51.
43. Park KS, Min K, Nam JH, Bang D, Lee ES, Lee S. Association of HYPA haplotype in the mannose-binding lectin gene-2 with Behcet's disease. Tissue Antigens **2005**; 65:260–5.
44. Holmberg V, Schuster F, Dietz E, et al. Mannose-binding lectin variant associated with severe malaria in young African children. Microbes Infect **2008**; 10:342–8.
45. Zanetti KA, Haznadar M, Welsh JA, et al. 3′-UTR and functional secretor haplotypes in mannose-binding lectin 2 are associated with increased colon cancer risk in African Americans. Cancer Res **2012**; 72:1467–77.
46. Koch A, Melbye M, Sorensen P, et al. Acute respiratory tract infections and mannose-binding lectin insufficiency in small children. Ugeskr Laeger **2002**; 164:5635–40.
47. Dunkelberger JR, Song WC. Complement and its role in innate and adaptive immune responses. Cell Res **2010**; 20:34–50.
48. Kilpatrick DC, Chalmers JD. Human L-ficolin (ficolin-2) and its clinical significance. J Biomed Biotechnol **2012**; 2012:138797.
49. Xu X, Stack RJ, Rao N, Caulfield JP. *Schistosoma mansoni*: fractionation and characterization of the glycocalyx and glycogen-like material from cercariae. Exp Parasitol **1994**; 79:399–409.
50. Hokke CH, Yazdanbakhsh M. Schistosome glycans and innate immunity. Parasite Immunol **2005**; 27:257–64.

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Lectin Complement Protein Collectin 11 (CL-K1) and Susceptibility to Urinary Schistosomiasis

Justin S. Antony[1], Olusola Ojurongbe[1,2], Peter G. Kremsner[1], Thirumalaisamy P. Velavan[1,3]*

1 Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany, **2** Ladoke Akintola University of Technology, Ogbomoso, Nigeria, **3** Fondation Congolaise pour la Recherche Medicale, Brazzaville, Republic of Congo

* velavan@medizin.uni-tuebingen.de

OPEN ACCESS

Citation: Antony JS, Ojurongbe O, Kremsner PG, Velavan TP (2015) Lectin Complement Protein Collectin 11 (CL-K1) and Susceptibility to Urinary Schistosomiasis. PLoS Negl Trop Dis 9(3): e0003647. doi:10.1371/journal.pntd.0003647

Editor: William Evan Secor, Centers for Disease Control and Prevention, UNITED STATES

Received: August 26, 2014

Accepted: February 25, 2015

Published: March 25, 2015

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This study was funded by an Institutional internal grant to TPV. OO was supported by Alexander von Humboldt foundation during the study period. The authors acknowledge the support by the Deutsche Forschungsgemeinschaft (DFG) and Open Access Publishing Fund of Tuebingen University. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background

Urinary Schistosomiasis is a neglected tropical disease endemic in many sub Saharan -African countries. Collectin Kidney 1 (CL-K1, encoded by *COLEC11* on chromosome 2p25.3), a member of the vertebrate C-type lectin super family, has recently been identified as pattern-recognition molecule (PRR) of the lectin complement pathway. CL-K1 is preferentially expressed in the kidneys, but also in other organs and it is considered to play a role in host defense to some infectious agents. Schistosome teguments are fucosylated and CL-K1 has, through its collagen-like domain, a high binding affinity to fucose.

Methodology/Principal Findings

We utilized a Nigerian study group consisting of 167 *Schistosoma haematobium* infected individuals and 186 matched healthy subjects, and investigated the contribution of CL-K1 deficiency and of *COLEC11* polymorphisms to infection phenotype. Higher CL-K1 serum levels were associated with decreased risk of schistosome infection (P^{corr} = 0.0004). CL-K1 serum levels were differentially distributed between the *COLEC11* genotypes and haplotypes observed. The non-synonymous variant *p.R216H* was associated with the occurrence of schistosomiasis (OR = 0.44, 95%CI = 0.22–0.72, P^{corr} = 0.0004). The reconstructed *COLEC11*TCCA* haplotypes were associated with higher CL-K1 serum levels (P = 0.002) and with decreased schistosomiasis (OR = 0.38, 95%CI = 0.23–0.63, P^{corr} = 0.0001).

Conclusions

In agreement with findings from our earlier published study, our findings support the observation that CL-K1 and their functional variants may be host factors associated with protection in schistosomiasis and may be a useful marker for further investigations.

Competing Interests: The authors have declared that no competing interests exist.

Author Summary

Collectin Kidney 1 (CL-K1) was discovered in 2006. It is a pattern-recognition molecule (PRR) of the lectin complement pathway and is mainly expressed in the kidneys. We investigated a possible functional role of CL-K1 during urinary schistosomiasis on the basis of the rationale: i). Schistosome teguments are fucosylated and CL-K1 has high binding affinity to fucose ii). As CL-K1 is structurally similar to MBL, we hypothesize the involvement of CL-K1 in immune modulation during urinary schistosomiasis. We investigated the circulating CL-K1 serum levels in our study group. We initially screened the entire promoter and the exons of the *COLEC11* gene for population specific variants. We validated four promoter variants and one non-synonymous substitution in exon8 for possible associations with urinary schistosomiasis and circulating CL-K1 serum levels. The study was conducted in Nigeria where the largest number of registered cases of schistosomiasis (29 million) for any sub-Saharan African country has been documented. Our findings support the observation that CL-K1 and functional variants are host factors that may be associated with protection in schistosomiasis and may be a useful marker for further investigations.

Introduction

Urogenital schistosomiasis, which is caused by infection with the trematode *Schistosoma haematobium*, is a major public health problem in sub-Saharan Africa (SSA). Of the more than 200 million cases reported worldwide, 93% occur in SSA [1]. Up to two-thirds of *S. haematobium* infections result in genital schistosomiasis [2]. The incidence of *S. haematobium* infections in SSA, however, is most likely underreported and might be much higher [3]. Schistosomiasis accounts for the loss of more than 70 million disability adjusted life years (DALYs) [4,5]. A large proportion of infected individuals experience hematuria (70 million), dysuria (32 million), bladder-wall pathology (18 million), and severe hydronephrosis (10 million) [6]. Urinary schistosomiasis is endemic in Nigeria and approximately 25 million people are currently infected, with an estimated 101 million at risk [7]. Schistosomiasis can also increase the risk of urinary tract infections and bladder cancer [8–10]. Children and early adolescents are at high risk of infection as their daily activities regularly include contact with water infested with infectious cercariae [11]. Limited access to praziquantel treatment for schistosomiasis, repeated re-exposure, and rapid reinfections all contribute to the disease burden [11,12].

Schistosomes are bisexual multicellular helminth parasites with six developmental stages including, adult worms, eggs, miracidia, sporocysts, cercariae and schistosomulae [13]. Schistosomes have an outer syncytial cytoplasmic layer, the tegument [14]. Previous studies have shown that the teguments consist of fucosylated carbohydrate epitopes (glycotopes) [15] and glycoproteins [16] which are expressed at all developmental stages. These glycoconjugates act as pathogen associated molecular patterns (PAMPs) that are recognized by pattern recognition molecules (PRMs) such as the C-type lectins [17]. Earlier *in vitro* studies have demonstrated successful complement-mediated tegument damage in the adult schistosomes [18]. We have previously shown that the lectin proteins mannose binding lectin (MBL) and MBL-associated serine protease 1 (MASP-1) and MASP-2 interact with schistosomal glycoconjugates, and subsequently activate the lectin complement cascade [19].

Collectin kidney 1 (CL-K1 also known as Collectin 11), is a member of the group of C-type lectins. The role of CL-K1 appears to be analogous to that of other C-type lectins [20]. To date, many studies have concentrated on the complement proteins MBL [21–24] and ficolins [25–28]

in disease. Far less is known about the mechanism of action of CL-K1. The human CL-K1 is encoded by *COLEC11* (OMIM 612502) on chromosome 2 at position 2p25.3 [29]. CL-K1 is a circulating serum protein and is expressed in many tissues. High mRNA expression is observed in kidneys, liver and in the adrenal glands. Similar to MBL, CL-K1 has a collagen like domain and a carbohydrate recognition domain (CRD) [20,30,31]. Six different genetic variants have been observed in a homozygous state in individuals affected with the rare Carnevale, Mingarelli, Malpuech and Michels (also known as 3MC) syndrome [32]. Two affected individuals with the p. Gly204Ser amino acid substitution in CRD of *COLEC11* had undetectable amounts of CL-K1 in their serum. Moreover, CL-K1 was shown to be a guidance cue for neural cell migration during embryogenesis [32]. The *COLEC11* variant rs10210631 is responsible for high IgE production in children [33]. CL-K1 recognizes pathogens by interacting with parasites' glycoconjugates [20,30]. A recent study has shown that CL-K1 can also deposit C4b upon binding with mannan in the presence of MASP-2 [34]. In addition, interactions of CL-K1 with MASP-1/3 have been well demonstrated [20,30].

We investigated the functional role of CL-K1 during urinary schistosomiasis on the basis of the following rationale: i). Schistosome teguments are fucosylated and CL-K1 has a high binding affinity to fucose [35] ii). as CL-K1 is structurally similar to MBL, we hypothesized that CL-K1 might be involved in immune modulation during urinary schistosomiasis. We therefore investigated CL-K1 serum levels in a study group of Nigerian individuals of Yoruba ethnicity as described in our previous studies [36,37]. Furthermore, we screened the entire *COLEC11* gene for population specific functional variants. We then evaluated four promoter variants and one non-synonymous substitution in *COLEC11* exon8 for associations with urinary schistosomiasis and circulating CL-K1 serum levels.

Materials and Methods

Ethics statement

Informed oral consent in the local language was obtained from all participants; for those who were children, informed consent was obtained from respective parents and/or guardians. The consent was verbal because the study was conducted in rural communities where the level of literacy was low. If the participants could not read or write, verbal consent was obtained after explaining the purpose of the study to them. The consent was written in a note book and only consenting individuals were recorded. The procedure was approved by the ethical committee of the Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Only those who provided their consent were recruited in the study. Ethical approval was also obtained from the ethical committee of Ministry of Health, Abeokuta Ogun State, Nigeria.

Study design

Two villages known to be endemic for *S. haematobium*, Ilewo Orile (Abeokuta North) and Ore (Osogbo), were chosen for the study. The communities lack sufficient clean water supply, safe waste disposal and essential health centers. Members of the communities depend on rivers in close proximity for their daily needs (collecting water, washing clothes, bathing). Fishing and petty trading are the most common occupations. Based on responses to a questionnaire, over 93% of the participants had regular water contact (defined as at least four contacts to the infected river in a week) for their daily needs. Most of the study participants had similar frequencies of exposure with water infested with infectious cercariae. Epidemiological studies of urinary schistosomiasis in Ogun state, Nigeria reported an infection rate of more than 80% [38]. The current study is a cross-sectional study and individuals were recruited blindly irrespective of their infection status. Individuals from all age groups who gave their consent to

participate in the study procedure were enrolled. Urine samples were collected from all individuals and were microscopically examined. Based on the results of the microscopic examination, the participants were divided into the case group positive for *S. haematobium* eggs in urine (SEP) and the negative control group. The control group was further screened to determine total anti-schistosoma IgG antibodies. Based on total IgG results, the control group was divided into two subgroups. The first subgroup contained individuals positive for anti-*Schistosoma* total IgG antibodies and negative for eggs in urine (SELP), and the second subgroup was negative for anti-*Schistosoma* total IgG antibodies and negative for eggs in urine (SELN). This classification is essential in endemic areas in order to differentiate individuals who were potentially resistant from those with a previous or a current infection. Therefore, the detection of anti-*Schistosoma* total IgG was employed as a marker of exposure.

Sample collection

Ten ml of urine were collected in a sterile container from all participants and the sample was centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded and the sediment was transferred to a clean glass slide which was microscopically examined for the presence of *S. haematobium* eggs. For negative individuals, urine samples were collected on three successive days in order to confirm that they were true negatives. A Combur-Test reagent strip (Roche Diagnostics GmbH Mannheim, Germany) was used according to the manufacturer's procedure to estimate the degree of haematuria and proteinuria. About 5 ml of blood sample was collected from all study participants for serological assays and subsequent DNA extraction. Those positive for urinary schistosomiasis were treated with a single dose of 40mg/kg praziquantel. Stool samples were collected from all participants and processed using the Kato-Katz method in order to exclude any individuals with *S. mansoni* infection.

Serological assays

Classification of the control group is essential in schistosoma endemic areas, as it is difficult to differentiate individuals without a current infection (either individuals are less susceptible or not been exposed to infection). Therefore detection of anti-schistosoma total IgG was employed as diagnostic exposure marker. Serological assays were carried out to determine the level of anti-*Schistosoma* total IgG antibodies in study participants' sera by an in-house ELISA assay. In brief: For each individual sample, eight wells were used. The *Schistosoma mansoni* adult antigen was serially diluted from 0.02 to 2.5 μg per 200μl in carbonate buffer ($NaHCO_3$ + Na_2CO_3 in water, pH = 9.6) and were pre-coated in each well. Negative and positive control plasma samples, were diluted 1:100 with milk buffer. 200μl of the test samples and controls were dispensed into the wells and were incubated for one hour at room temperature. After incubation and subsequent washing steps, each well was treated with 200μl of conjugate solution [goat anti-human IgG bound to alkaline phosphatase, (Sigma-Aldrich, Munich, Germany)] at a concentration of 1:10,000 in 1% milk buffer and incubated for one hour at room temperature. After incubation and subsequent washing steps (5x), 200μl of the substrate [pNPP: 4- Nitrophenyl phosphate disodium salt hexahydrate (Sigma-Aldrich, Munich, Germany)] was added to each well and further incubated for 15 minutes at room temperature. The optical density (OD) was measured at 405 nm. CL-K1 serum levels were determined in all study subjects in 1:5 diluted plasma by a commercially available CL-K1 ELISA kit ELISA kit (EIAab Science, Taiwan) following the manufacturer's instruction. The lower detection limit of the assay was 7.8 ng/ml.

COLEC11 genotyping

Genomic DNA was extracted from blood cells using the QIAamp DNA mini blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. In a first step, the *COLEC11* gene was screened by amplifying the promoter and the eight exons including intron-exon boundaries in 65 healthy individuals. A total of nine genomic fragments of the entire *COLEC11* promoter region, including the eight exons were amplified using 10 ng of genomic DNA. The PCR mix consisted of 1x PCR buffer (20 mM Tris-HCl, pH 8.4; 50 mM KCl, 2 mM $MgCl_2$), 0.125 mM of dNTPs, 2 μM of sequence-specific primer pairs and 1 U Taq DNA polymerase (Qiagen, Hilden, Germany). A PTC-200 Thermal cycler (MJ Research, USA) was used. Primer sequences and thermal cycling parameters for the nine PCR reactions are listed in the S1 Table. Subsequently, PCR products were purified (Exo-SAP-IT; USB, Affymetrix, USA) and 1 μl of the product was used as template for DNA-sequencing (BigDye terminator v.1.1 cycle sequencing kit, Applied Biosystems, USA) on an ABI 3130XL sequencer. Sequences were aligned with the reference sequence of the *COLEC11* gene (NCBI; NG_031954.1) using the CodonCode Aligner 4.0 software (http://www.codoncode.com/) and confirmed visually from their electropherograms.

Based on observed frequencies of $\geq$ 10% of *COLEC11* variants and on variants with recognized functional significance, the variants subjected to further investigation were selected. Four promoter variants (−676T>C, −472T>C, −469C>G, −276C>T), and the non-synonymous (ns) substitution p.R216H in exon8 were genotyped in the entire study group using the primer pairs and PCR conditions as described in S2 Table.

Statistical analysis

Data were analyzed using the STATA software (STATA Corp., College Station, TX, USA) and the level of significance was set to a p-value of <0.05. Kruskal Wallis rank sum tests following Dunn's multiple comparison post test were used to analyze the correlation of serum CL-K1 levels with distinct *COLEC11* variants using Graphpad Prism v6.0. Fisher's exact test and logistic regression analyses after adjustment for age and gender were performed to examine associations of CL-K1 variants with schistosomiasis. Correlation analyses were performed by non-parametric Spearman´s rank coefficient tests as implemented in Graphpad Prism v.6.0. Genotype and haplotype frequencies were analyzed by gene counting and expectation-maximum (EM) algorithms and the significance of deviation from Hardy-Weinberg equilibrium was tested using the random-permutation procedure as implemented in the Arlequin v. 3.5.1.2 software (http://lgb.unige.ch/arlequin). Linkage disequilibrium (LD) analysis was performed using Haploview v. 3.2 (http://broadinstitute.org/haploview).

Results

Characteristics of the study groups

After parasitological and serological tests, our study group was divided into three groups. The case group (SEP) was defined as being positive for *Schistosoma haematobium* eggs in urine [(n = 167), 100(60%) children, 93(55%) males, 74(45%) females and the mean age 17.5 ±13.2]. The first control group is defined as negative for *S. haematobium* eggs in urine but positive for anti-schistosoma total IgG antibodies (SELP) [(n = 119) 22(19%) children, 60(50%) males, 59 (50%) females and the mean age 34.3±19.1] and the second control group defined as negative for *S. haematobium* egg in urine and also negative for anti-schistosoma total IgG antibodies (SELN) [(n = 67), 33(49%) children, 41(61%) males, 26(39%) females and the mean age 20.4 ±17.1]. All of the study subjects belong to the Yoruba ethnicity of the Nigerian population.

The epidemiological data for the case control groups were: Mean age (SEP = 17.5 [4–71], SELP = 34[4–75], SELN = 20[4–71]) and hematuria (SEP = 90%, SELP = 2.4%, SELN = 0%), respectively. The mean parasite count for individuals in the SEP group was 1595 (20–27000) per 10 ml urine.

COLEC11 gene polymorphisms

A two step approach was followed to obtain population specific frequencies of *COLEC11* variants in the investigated study group. First, the entire promoter region and the eight exons including the intron-exon boundaries were screened in healthy controls (SELN n = 67). The observed frequencies of variants was compared with available HapMap data (S2 Table). The LD plot of observed variants in the entire *COLEC11* gene is given in S1 Fig. In the second step, all polymorphisms in the promoter region and the non-synonymous variant p.R216H in exon8 were chosen for further genotyping in the entire study group for investigation of genetic associations with infection phenotype.

COLEC11 gene polymorphisms and *S. haematobium* infection risk

The four gene variants in the promoter region, −676T>C, −472T>C, −469C>G, −276C>T, and the non-synonymous substitution p.R216H in exon8 were genotyped. LDs of the five *COLEC11* variants in the case group and the two control subgroups are illustrated in Fig. 1. The promoter variants rs1864480 (−676T/C) and rs4849953 (−472T/C) were observed to be in strong LD in all groups. Genotype and allele frequencies in all groups were in Hardy-Weinberg equilibrium, except for variant rs1864480 (−676T/C) in the egg positive (SEP) cases.

The non-synonymous *COLEC11* variant rs7567833G/A (p.R216H) in exon8 was observed more frequently among infected individuals (SEP) than egg and IgG-negative (SELN) healthy controls. The *COLEC11* homozygous genotype of the major allele rs7567833-GG was observed significantly more often in the SEP group compared to SELN controls after adjusting for age and gender (OR = 2.35, 95%CI = 1.26–4.37,P^{corr} = 0.004), suggesting an association with increased risk of infection. The *COLEC11* homozygous genotype of the minor allele rs7567833-AA was observed significantly less often in the SEP group than in the SELN group (OR = 0.2, 95% CI = 0.08–0.90,P^{corr} = 0.01), showing an association with decreased risk of urinary schistosomiasis. Similar effects were also observed in the allele distributions (allelic model: OR = 0.44, 95% CI = 0.22–0.72, P^{corr} = 0.0004; dominant model: OR = 0.42, 95%CI = 0.22–0.79, P^{corr} = 0.0048; and recessive model: OR = 0.2, 95%CI = 0.08–0.9, P^{corr} = 0.01) (Table 1). The observations from the different models indicate a significant contribution of the non-synonymous p.216H substitution as a host genetic factor predisposing to schistosomiasis. Variant p.216H was not observed in linkage with any other *COLEC11* variant (Fig. 1). The other investigated *COLEC11* variants −472T>C, −469C>G, −276C>T were not associated with urinary schistosomiasis.

The distribution of the reconstructed *COLEC11* haplotypes including −472T>C, −469C>G, −276C>T and +48912G>A are summarized in Table 2. Six haplotypes associated with circulating levels of CL-K1 were observed. Among them, *COLEC11*TCCG*, *COLEC11*CCCG* and *COLEC11*TCCA* were observed at higher frequencies in the entire study group. The *COLEC11*TCCG* haplotype, representing all major alleles, was observed more frequently in cases than in SELN controls (OR = 1.76, 95%CI = 1.15–2.70, P^{corr} = 0.007). *COLEC11*TCCA*-p.R216H, was observed more frequently among SELN controls (SEP vs. SELN: OR = 0.38, 95%CI = 0.23–0.63, P^{corr} = 0.0001; SEP vs. SELP+SELN controls: OR = 0.66, 95%CI = 0.43–0.99, P^{corr} = 0.04).

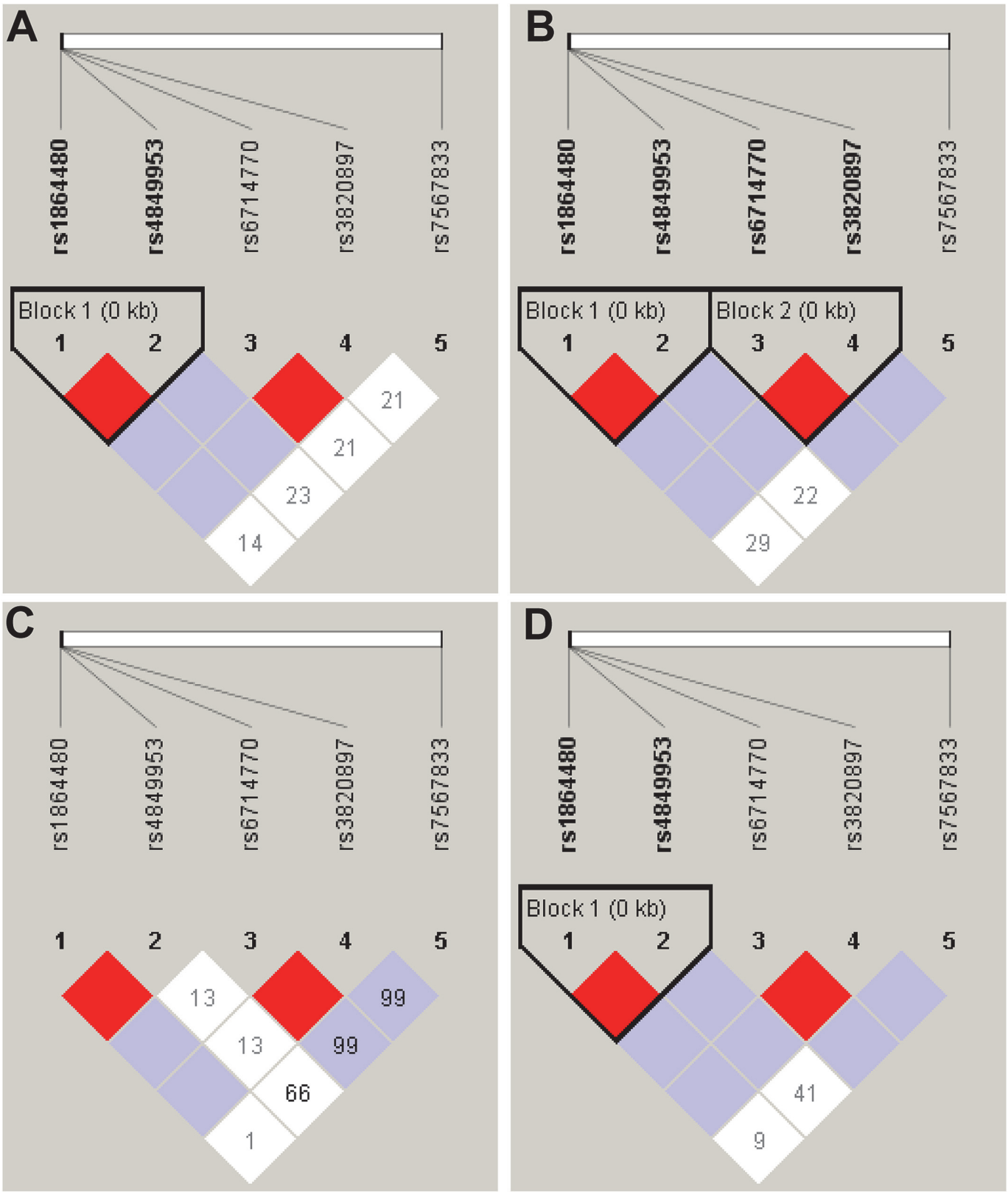

Fig 1. Linkage disequilibrium (LD) pattern of *COLEC11* variants in SEP cases group (A), in SELP control group (B), in SELN control group (C) and in SELP+SELN combined control group (D). Open white squares indicate a high degree of LD (D' = 1) between pairs of markers. Numbers indicate the D'

value expressed as a percentile. The red square indicates pairs in strong LD with LOD scores ≥ 2; purple squares, D' = 1 with LOD scores ≤ 1. The haplotype block is outlined by a solid line.

doi:10.1371/journal.pntd.0003647.g001

CL-K1 serum levels and *S. haematobium* infection risk

Mean circulating CL-K1 serum levels among Nigerian individuals without schistosomiasis were 246±155 ng/mL, largely similar to levels observed in Japanese (340±130 ng/mL), Danish (284± ~ 180 ng/mL) and American populations (265±177 ng/mL) [25, 26, 35]. The median circulating CL-K1 serum levels in SEP, SELN and SELP+SELN were 161 ng/ml, 206 ng/ml and 175 ng/ml, respectively. Circulating CL-K1 serum levels were heterogeneously distributed between our study subgroups ($P = 0.0007$) (SEP vs. SELN, $P<0.001$; SEP vs. SELP+SELN, $P>0.05$) (Fig. 2).

Association of *COLEC11 variants* to circulating CL-K1 serum levels

The minor allele of exon8 variant rs7567833A (p.R216H) was significantly associated with increased CL-K1 serum levels (Fig. 3A and Fig. 3B). A gene dose-dependent effect on the distribution of serum CL-K1 levels was observed. Individuals with the COLEC11*TCCA haplotype had higher CL-K1 serum levels in both control groups (SELN: $P = 0.01$ and SELP+SELN: $P = 0.0004$) (Fig. 4), but such a trend was not observed in egg positive (SEP) individuals (S2 Fig). When only COLEC11*TCCA haplotypes were compared among the investigated groups, the SEP group had lower CL-K1 serum levels than the groups (SELP, SELP+SELN) (P<0.0001) (Fig. 5). The *COLEC11* haplotypes may further be classified as high expression (*COLEC11*CCCG* + *COLEC11*TCCA*) or as low expression (*COLEC11*TCCG*) haplotypes based on circulating serum CL-K1 levels observed in the control subgroups (Table 2). The low secretor haplotype (*COLEC11*TCCG*) was associated

Table 1. Distribution of *COLEC11*—rs7567833G/A (*p.R216H*) genotypes and allele(s).

	Genotype	SEP[a] n = 167 (%)	SELP[b] n = 119 (%)	SELN[c] n = 67 (%)	SELP[b]+SELN[c] n = 186 (%)	SEP[a] vs. SELN[c] OR (95% CI)	*p*[#]value
Exon8 rs7567833 G/A (p. R216H)	GG	114 (68.2)	81 (68)	32 (47.8)	113 (60.7)	2.35 (1.26–4.37)	0.004
	GA	46 (27.5)	37 (31)	26 (38.8)	63 (33.9)		
	AA	7 (4.3)	1 (1)	9 (13.4)	10 (5.4)	0.2 (0.08–0.9)	0.01
	Allele						
	G	274 (82)	199 (83.6)	90 (67.2)	289 (77.7)	**Reference**	
	A	60 (18)	39 (16.4)	44 (32.8)	83 (22.3)	0.44 (0.27–0.72)	0.0008
	Dominant						
	GG	114 (68.2)	81 (68)	32 (47.8)	113 (60.7)	**Reference**	
	GA+AA	53(31.8)	38(32)	35(52.2)	73 (39.3)	0.42 (0.22–0.79)	0.004
	Recessive						
	GG+GA	160 (95.7)	118 (99)	58 (86.4)	176 (94.6)	**Reference**	
	AA	7 (4.3)	1 (1)	9 (13.4)	10 (5.4)	0.2 (0.08–0.9)	0.01

Note. CI, confidence interval; OR, odds ratio.

Percentage may not add up to 100 due to rounding errors

[#] Adjusted *P* values for age and gender

[a] diagnosed with *S. haematobium* egg in urine [SEP]

[b] Negative for *S. haematobium* egg in urine but positive for anti-schistosoma total IgG [SELP]

[c] Negative for *S. haematobium* egg and anti-schistosoma total IgG [SELN]

doi:10.1371/journal.pntd.0003647.t001

Table 2. Distribution of *COLEC11* haplotypes in the investigated study groups

*COLEC11** Haplotypes	SEP[a] (n = 334)	SELP[b] (n = 238)	SELN[c] (n = 134)	SELP[b] + SELN[c] (n = 372)	SEP[a] vs SELN[c] OR (95% CI)	*p*[#] value
*COLEC11*TCCG*	184 (55)	142 (60)	55 (41)	197 (53)	1.76(1.15–2.70)	0.007
*COLEC11*CCCG*	83 (24.9)	45 (19)	33 (24.6)	78 (21)		NS
*COLEC11*TCCA*	50 (15)	36 (15)	42 (31.4)	78 (21)	0.38(0.23–0.63)	0.0001
*COLEC11*TGTG*	7 (2)	12(5)	2 (1.5)	14 (3.5)		NS
*COLEC11*TGTA*	6 (1.8)	0 (0)	0 (0)	0 (0)		NS
*COLEC11*CCCA*	4 (1.3)	3(1)	2 (1.5)	5 (1.5)		NS
Low Expression						
*COLEC11*TCCG*	184 (55)	142 (60)	55 (41)	197 (53)	1.76(1.15–2.70)	0.007
High Expression						
*COLEC11*CCCG + COLEC11*TCCA*	133 (39.8)	81 (34)	75 (56)	156 (42)	0.52(0.33–0.79)	0.002

Note. CI, confidence interval; OR, odds ratio.

Percentage may not add up to 100 due to rounding errors

[#] Adjusted *P* values for age and gender

[a] diagnosed with *S. haematobium* egg in urine [SEP]

[b] Negative for *S. haematobium* egg in urine but positive for anti-schistosoma total IgG [SELP]

[c] Negative for *S. haematobium* egg and anti-schistosoma total IgG [SELN]

doi:10.1371/journal.pntd.0003647.t002

with *S. haematobium* infection (OR = 1.76, 95%CI = 1.15–2.70, P^{corr} = 0.007) and the high secretor haplotypes were associated with decreased risk of infection (OR = 0.52, 95%CI = 0.33–0.79, P^{corr} = 0.002).

Discussion

Different immune strategies are employed by the host immune system to thwart an infection and the innate immune system plays a critical role in the clearance of some pathogens. Immune evasion from complement components is an important criterion for schistosomes to successfully establish an infection [14,39]. Lectin pathway proteins of the complement system are the first components to recognize the pathogen. These proteins can initiate a complement attack cascade independent of a specific antibody response [40]. Our previous studies have demonstrated that lectin proteins Ficolin-2 [37] and MBL [36] are involved in *S. haematobium* infections.

CL-K1 was first described in 2006 but relatively few studies only have looked at its role in infectious diseases. This study investigated the association between serum CL-K1 level and functional genetic variants in the *COLEC11* gene in urinary schistosomiasis. Our study suggests that a non-synonymous substitution p.R216H in the exon8 of *COLEC11* contributes to susceptibility to schistosomiasis. In particular, the major allele p.R216 increases the infection risk twofold compared to variant p.216H. Similar effects were also observed in different genetic models for the contribution of the respective p.R216H genotypes. It has been hypothesized that the p.R216H substitution increases the alpha helical propensity value that controls the protein stability and protein folding properties [41]. The p.R216H substitution is located in the carbohydrate recognition domain (CRD) of *COLEC11*, and therefore binding of the CRD with schistosome elements may be impaired. In addition, this particular variant rs7567833G/A (p.R216H) was reported to be under selective pressure [42] and was differentially distributed among a panel of 52 populations as described in HapMap and in the Human Genome Diversity Project–Centre

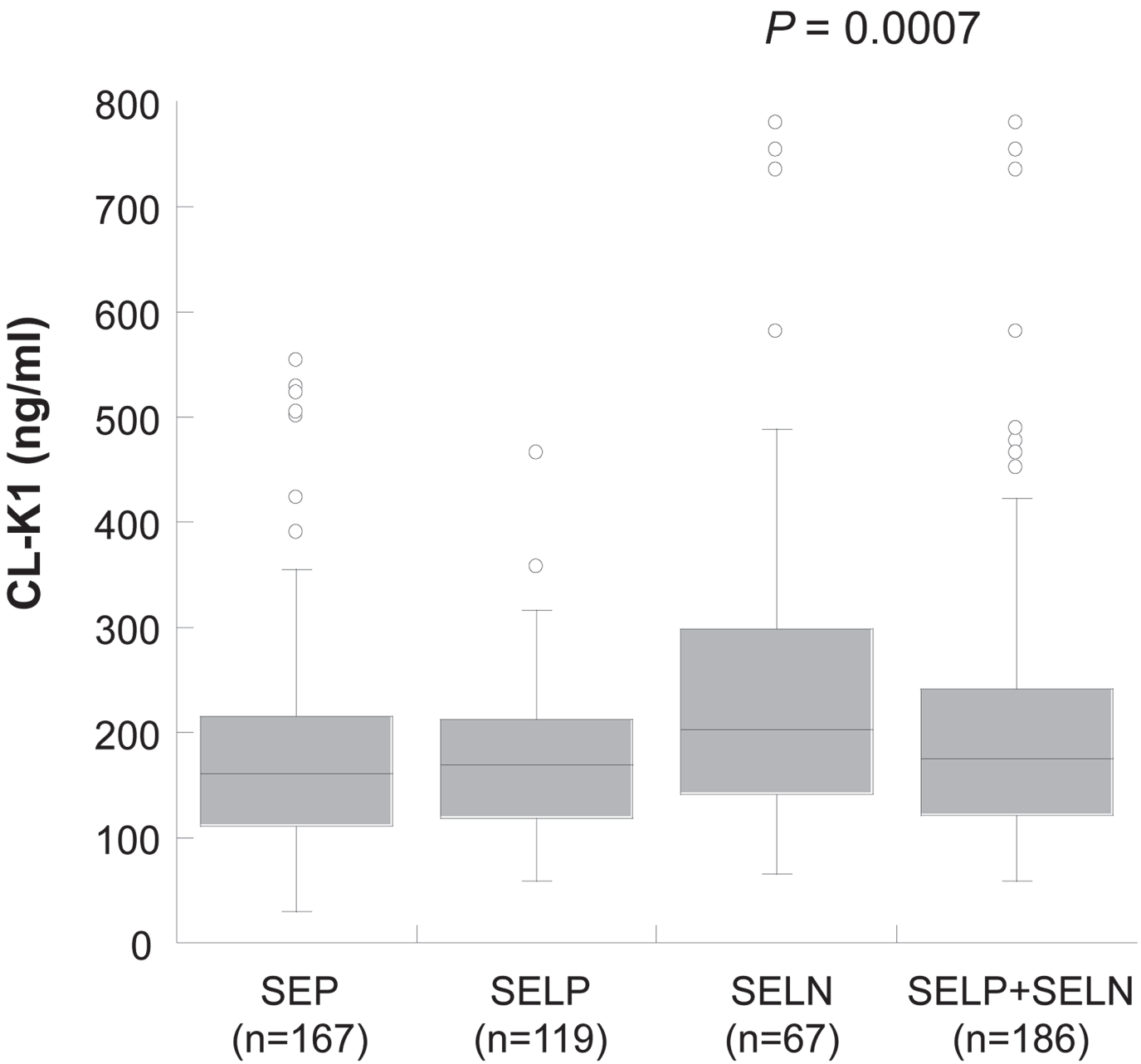

Fig 2. Distribution of CL-K1 serum levels (median values) among the study groups (SEP: diagnosed with *S. haematobium* egg in urine; SELP: Negative for *S. haematobium* egg in urine but positive for anti-schistosoma total IgG; SELN: Negative for *S. haematobium* egg and anti-schistosoma total IgG). $P = 0.007$ illustrated in the figure is calculated by Kruskal-Wallis rank sum test. Study group comparison were calculated by Dunn's multiple comparison post test (SEP vs. SELN, $P<0.001$; SEP vs. SELP+SELN, $P>0.05$). Numbers in parentheses indicates absolute counts of sample size in each group.

doi:10.1371/journal.pntd.0003647.g002

d'Etude du Polymorphisme Humain (HGDP–CEPH) databases [43]. Also, the absence of LD with other genetic variants in proximity indicates selection. None of the promoter polymorphisms contributed to schistosomiasis susceptibility. In addition, the regulatory polymorphisms in the promoter region does not appear to play a role in CL-K1 expression as reported in another study [44].

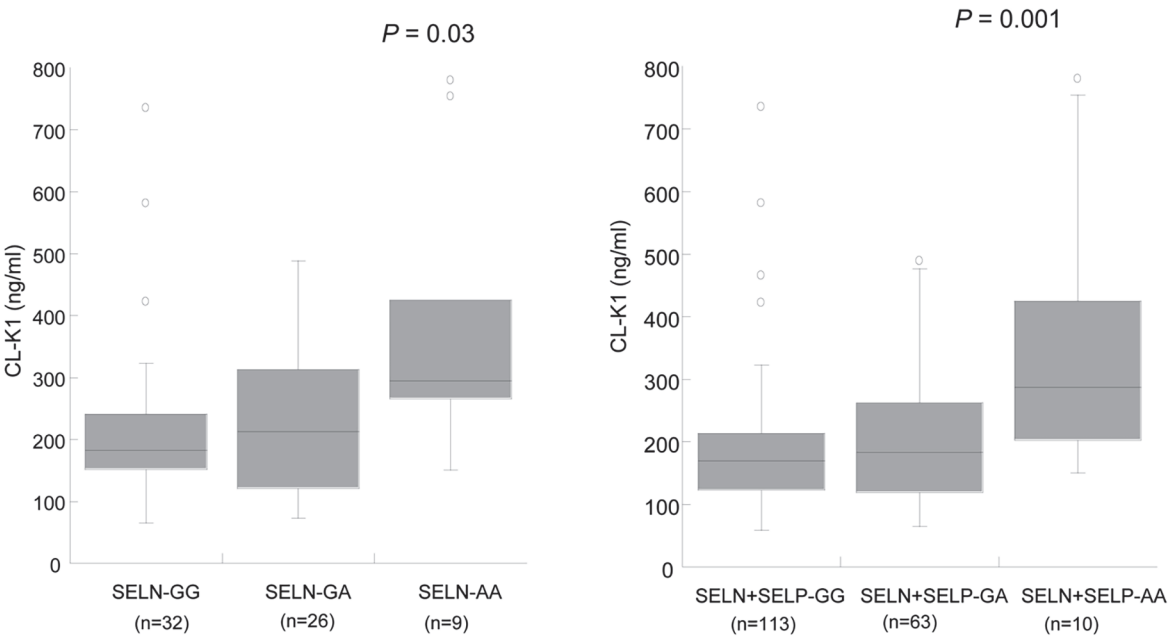

Fig 3. Distribution of CL-K1 serum levels (median values) with investigated rs7567833G/A (*p.R216H*) variant. (Left) SELN group (Right): SELP+SELN group. $P = 0.03$ and $P = 0.001$ illustrated in the figures are calculated by Kruskal-Wallis rank sum test. Study group comparison were calculated by Dunn's multiple comparison post test (SELN-GG vs. SELN-AA, $P<0.05$; SELN-GA vs. SELN-AA, $P>0.05$); (SELN+SELP-GG vs. SELN+SELP-AA, $P<0.01$; SELN+SELP-GA vs. SELN+SELP-AA, $P<0.05$). Numbers in parentheses indicates absolute counts of sample size in each group. (**SEP**: diagnosed with *S. haematobium* egg in urine; **SELP**: Negative for *S. haematobium* egg in urine but positive for anti-schistosoma total IgG; **SELN**: Negative for *S. haematobium* egg and anti-schistosoma total IgG).

doi:10.1371/journal.pntd.0003647.g003

Stratification of our study group based on reconstructed *COLEC11* haplotypes revealed that the frequency of the *COLEC11*TCCG* haplotype was significantly more frequent in the infection group than in controls, suggesting that individuals with these haplotypes had a higher risk of schistosomiasis. In addition, the *COLEC11*TCCA* haplotype harboring the rs7567833-A polymorphism occurred more frequently in healthy controls compared to the infection group, suggesting that individuals with this haplotype were protected from *S. haematobium* infection. Furthermore, when the SELP plus SELN controls were analyzed, significant differences were observed with the same haplotype, supporting the suggestion that the *COLEC11*TCCA* haplotype may help confer protection. The frequency of high CL-K1 expressing haplotypes was higher in controls than in SEP cases.

This study demonstrates that CL-K1 serum levels were higher in the control group compared with infected individuals, suggesting that high levels of CL-K1 might reduce the risk of *S. haematobium* infection. Similar to MBL and ficolins, CL-K1 could recognize and bind to specific glycoproteins on the surface of the pathogens [7,13]. In line with our earlier studies on Ficolin-2 [37] and MBL [36], we believe that CL-K1 serum levels may be down regulated during *S. haematobium* infection. Recent investigations in patients with disseminated intravascular coagulation (DIC) have shown that CL-K1 levels were significantly elevated [44]. The *COLEC11* rs7567833G/A (p.R216H) variant was observed to correlate with increased CL-K1 serum levels. In addition, the *COLEC11*TCCA* haplotype with the allele *p.216H* was associated with higher CL-K1 serum levels in healthy individuals. Inversely, *COLEC11*TCCG* with allele *p.R216* was associated with lower CL-K1 serum levels. These results substantiate that the variant in exon8 is a host genetic factor that may help protect against schistosomiasis. When the individuals with *COLEC11*TCCA* were analyzed for CL-K1 serum levels in the different patient

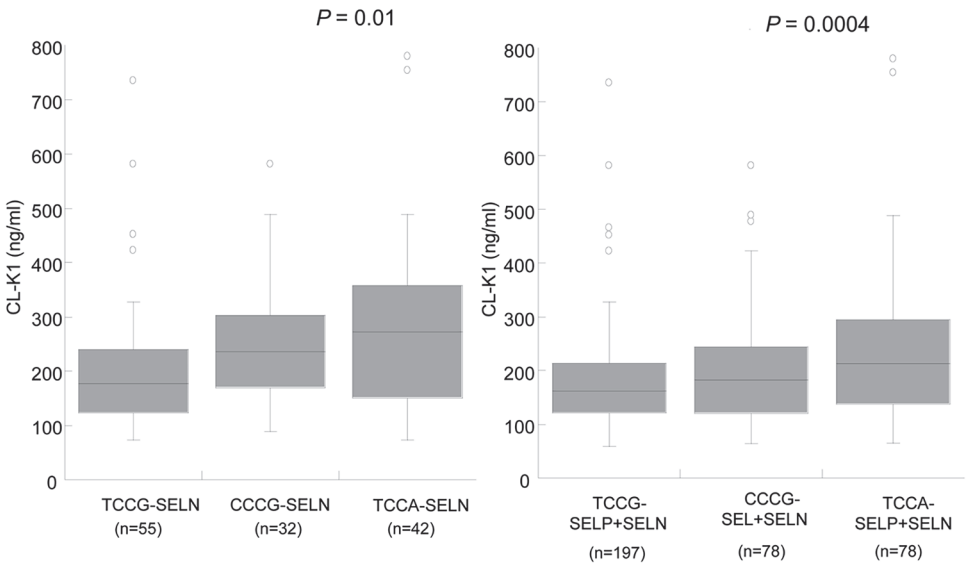

Fig 4. Distribution of CL-K1 serum levels (median values) with investigated *COLEC11* haplotypes (Left) SELN controls (Right): SELP+SELN combined controls. $P = 0.03$ and $P = 0.0004$ illustrated in the figures are calculated by Kruskal-Wallis rank sum test. Study group comparison were calculated by Dunn's multiple comparison post test (*COLEC11*TCCG*-SELN vs. *COLEC11*TCCA*-SELN, $P<0.05$; *COLEC11*CCCG*-SELN vs. *COLEC11*TCCA*-SELN, $P>0.05$; *COLEC11*TCCG*-SELN+SELP vs. *COLEC11*TCCA*-SELN+SELP, $P<0.001$; *COLEC11*TCCG*-SELN+SELP vs. *COLEC11*CCCG*-SELN+SELP, $P>0.05$). Numbers in parentheses indicate absolute counts of sample size in each group. **SEP**: diagnosed with *S. haematobium* egg in urine; **SELP**: Negative for *S. haematobium* egg in urine but positive for anti-schistosoma total IgG; **SELN**: Negative for *S. haematobium* egg and anti-schistosoma total IgG).

doi:10.1371/journal.pntd.0003647.g004

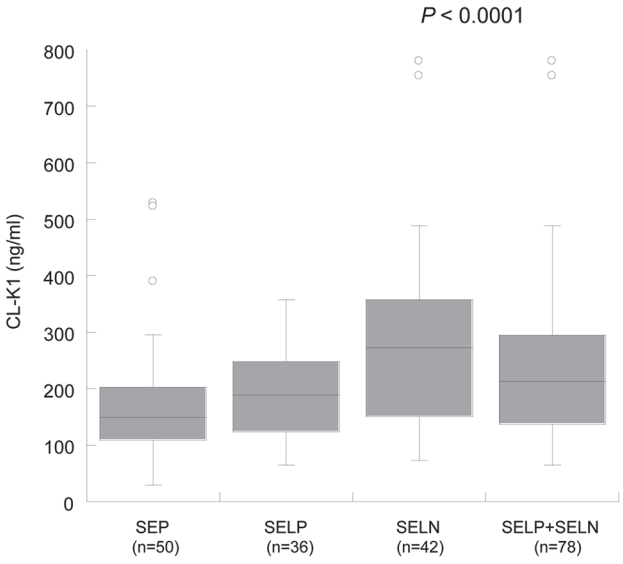

Fig 5. Distribution of CL-K1 serum levels (median values) with investigated *COLEC11*TCCA* haplotype in all study groups. $P<0.0001$ illustrated in the figure is calculated by Kruskal-Wallis rank sum test. Study group comparison were calculated by Dunn's multiple comparison post test (*COLEC11*TCCA*-SEP vs. *COLEC11*TCCA*-SELN, $P<0.001$; *COLEC11*TCCA*-SEP vs. *COLEC11*TCCA*-SELP+SELN, $P<0.01$). Numbers in parentheses indicates absolute counts of sample size in each group. **SEP**: diagnosed with *S. haematobium* egg in urine; **SELP**: Negative for *S. haematobium* egg in urine but positive for anti-schistosoma total IgG; **SELN**: Negative for *S. haematobium* egg and anti-schistosoma total IgG.

doi:10.1371/journal.pntd.0003647.g005

groups, SEP individuals had lower levels than egg negatives, indicating that CL-K1 serum levels were modulated by infection.

CL-K1 has a collagen and a ligand binding domains, similar to MBL and ficolins. These structural domains bind specifically to pathogen-associated molecular patterns (PAMPs) on the surface of infectious agents [45]. We postulate that CL-K1, along with the MBL-associated serine proteases MASP-1/3 and MASP-2 initiate the complement lectin cascade to reduce *S. haematobium* infection. [20,30,34].

We have shown that allelic variants associated with increased CL-K1 levels may be a contributing protective host factor in schistosomiasis caused by *S. haematobium*. Furthermore, the variant in *p.R216H* in exon8 of the *COLEC11* gene is a host genetic factor associated with urinary schistosomiasis. Taken together, both *COLEC11* variants and CL-K1 serum levels are associated with the phenotype occurring after *S. haematobium* infection.

Supporting Information

S1 Checklist. STROBE checklist.
(DOCX)

S1 Table. Primer pairs and PCR program conditions utilized for screening the COLLEC11 gene.
(DOCX)

S2 Table. Distribution of screened COLEC11 variants in SELN Controls and its comparison with HapMap data of Yoruba ethnicity.
(DOCX)

S1 Fig. Linkage disequilibrium pattern of screened COLEC11 variants in SELN control group. Open white squares indicate a high degree of LD (D' = 1) between pairs of markers. Numbers indicate the D' value expressed as a percentile. The red square indicates pairs in strong LD with LOD scores ≥ 2; purple squares, D' = 1 with LOD scores ≤ 1. A solid line outlines the haplotype block.
(TIF)

S2 Fig. Distribution of CL-K1 serum levels (median values) with investigated COLEC11 haplotypes in SEP cases (SEP: diagnosed with S. haematobium egg in urine). P = 0.8 value illustrated in the figure is calculated by Kruskal-Wallis rank sum test. Numbers in parentheses indicates absolute counts of sample size in each group.
(TIF)

Acknowledgments

We thank Abass Busari of State Hospital Ijaye, Abeokuta, Sanusi Tawakalitu. Olaolu Abdulazeez and Adedokun Samuel of Ladoke Akintola University of Technology, Osogbo for the technical support. We extend our thanks to Akeem Akindele, Olawumi Sina-Agbaje and Adegboyega Adeyeba for their extensive help during sampling. We also thank all the staff of Schistosomiasis control unit, Ministry of Health, Abeokuta, and the community members for their participation in the study. We thank Prabhanjan Gai for help with sequencing.

Author Contributions

Conceived and designed the experiments: TPV JSA. Performed the experiments: JSA. Analyzed the data: JSA TPV. Contributed reagents/materials/analysis tools: TPV PGK OO. Wrote the paper: TPV JSA OO.

References

1. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J (2006) Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. The Lancet infectious diseases 2006/06/23: 411–425. Meta-Analysis Research Support, Non-U.S. Gov't Review.
2. Hotez PJ, Fenwick A, Kjetland EF (2009) Africa's 32 cents solution for HIV/AIDS. PLoS neglected tropical diseases 2009/05/30: e430. Editorial.
3. King CH (2010) Parasites and poverty: the case of schistosomiasis. Acta tropica 2009/12/08: 95–104. Research Support, N.I.H., Extramural Review.
4. King CH, Dickman K, Tisch DJ (2005) Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. Lancet 2005/05/04: 1561–1569. Meta-Analysis Research Support, U.S. Gov't, P.H.S.
5. King CH, Dangerfield-Cha M (2008) The unacknowledged impact of chronic schistosomiasis. Chronic illness 2008/03/07: 65–79. Research Support, N.I.H., Extramural Review.
6. Ekpo UF, Laja-Deile A, Oluwole AS, Sam-Wobo SO, Mafiana CF (2010) Urinary schistosomiasis among preschool children in a rural community near Abeokuta, Nigeria. Parasit Vectors 3: 58. doi: 10.1186/1756-3305-3-58 PMID: 20602792
7. Hotez PJ, Asojo OA, Adesina AM (2012) Nigeria: "Ground Zero" for the high prevalence neglected tropical diseases. PLoS neglected tropical diseases 2012/08/04: e1600. Editorial.
8. Bamgbola OF (2014) Urinary schistosomiasis. Pediatric nephrology 2014/01/29.
9. Botelho MC, Machado JC, Brindley PJ, Correia da Costa JM (2011) Targeting molecular signaling pathways of Schistosoma haemotobium infection in bladder cancer. Virulence 2011/07/27: 267–279. Review.
10. van der Werf MJ, de Vlas SJ, Brooker S, Looman CW, Nagelkerke NJ, Habbema JD, Engels D (2003) Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. Acta tropica 2003/05/15: 125–139. Research Support, Non-U.S. Gov't.
11. Stothard JR, Sousa-Figueiredo JC, Betson M, Bustinduy A, Reinhard-Rupp J (2013) Schistosomiasis in African infants and preschool children: let them now be treated! Trends in parasitology 2013/03/08: 197–205. Research Support, Non-U.S. Gov't Review.
12. Yazdanbakhsh M, Sacks DL (2010) Why does immunity to parasites take so long to develop? Nature reviews Immunology 2010/02/26: 80–81.
13. Ross AG, Bartley PB, Sleigh AC, Olds GR, Li Y, Williams GM, McManus DP (2002) Schistosomiasis. The New England journal of medicine 2002/04/19: 1212–1220. Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review.
14. Skelly PJ, Alan Wilson R (2006) Making sense of the schistosome surface. Advances in parasitology 2006/12/01: 185–284. Review.
15. Peterson NA, Hokke CH, Deelder AM, Yoshino TP (2009) Glycotope analysis in miracidia and primary sporocysts of Schistosoma mansoni: differential expression during the miracidium-to-sporocyst transformation. International journal for parasitology 2009/06/24: 1331–1344. Research Support, N.I.H., Extramural.
16. Hayunga EG, Sumner MP (1986) Expression of lectin-binding surface glycoproteins during the development of Schistosoma mansoni schistosomula. The Journal of parasitology 1986/12/01: 913–920. Research Support, U.S. Gov't, P.H.S.
17. Meevissen MH, Yazdanbakhsh M, Hokke CH (2012) Schistosoma mansoni egg glycoproteins and C-type lectins of host immune cells: molecular partners that shape immune responses. Experimental parasitology 2011/05/28: 14–21. Review.
18. Rasmussen KR, Kemp WM (1987) Schistosoma mansoni: interactions of adult parasites with the complement system. Parasite immunology 1987/03/01: 235–248. Research Support, Non-U.S. Gov't.
19. Klabunde J, Berger J, Jensenius JC, Klinkert MQ, Zelck UE, Kremsner PG, Kun JF (2000) Schistosoma mansoni: adhesion of mannan-binding lectin to surface glycoproteins of cercariae and adult worms. Experimental parasitology 2000/10/20: 231–239. Research Support, Non-U.S. Gov't.

20. Keshi H, Sakamoto T, Kawai T, Ohtani K, Katoh T, Jang SJ, Motomura W, Yoshizaki T, Fukuda M, Koyama S, Fukuzawa J, Fukuoh A, Yoshida I, Suzuki Y, Wakamiya N (2006) Identification and characterization of a novel human collectin CL-K1. Microbiology and immunology 2006/12/21: 1001–1013. Research Support, Non-U.S. Gov't.

21. Bouwman LH, Roep BO, Roos A (2006) Mannose-binding lectin: clinical implications for infection, transplantation, and autoimmunity. Human immunology 2006/05/25: 247–256. Review.

22. Goeldner I, Skare TL, Utiyama SR, Nisihara RM, Tong H, Messias-Reason IJ, Velavan TP (2014) Mannose binding lectin and susceptibility to rheumatoid arthritis in brazilian patients and their relatives. PLoS One 9: e95519. doi: 10.1371/journal.pone.0095519 PMID: 24751721

23. Jha AN, Sundaravadivel P, Singh VK, Pati SS, Patra PK, Kremsner PG, Velavan TP, Singh L, Thangaraj K (2014) MBL2 variations and malaria susceptibility in Indian populations. Infect Immun 82: 52–61. doi: 10.1128/IAI.01041-13 PMID: 24126531

24. Velavan TP, Boldt AB, Tomiuk J, Seibold F, Schoepfer AM, Flogerzi B, Muller S, Abad-Grau MM, Kremsner PG, Kun JF (2010) Variant alleles of the mannose binding lectin 2 gene (MBL2) confer heterozygote advantage within Crohn's families. Scand J Gastroenterol 45: 1129–1130. doi: 10.3109/00365521.2010.485324 PMID: 20443743

25. Ren Y, Ding Q, Zhang X (2014) Ficolins and infectious diseases. Virologica Sinica 2014/01/24: 25–32.

26. Assaf A, Hoang TV, Faik I, Aebischer T, Kremsner PG, Kun JF, Velavan TP (2012) Genetic evidence of functional ficolin-2 haplotype as susceptibility factor in cutaneous leishmaniasis. PLoS One 7: e34113. doi: 10.1371/journal.pone.0034113 PMID: 22457818

27. Hoang TV, Toan NL, Song lH, Ouf EA, Bock CT, Kremsner PG, Kun JF, Velavan TP (2011) Ficolin-2 levels and FCN2 haplotypes influence hepatitis B infection outcome in Vietnamese patients. PLoS One 6: e28113. doi: 10.1371/journal.pone.0028113 PMID: 22140517

28. Luz PR, Boldt AB, Grisbach C, Kun JF, Velavan TP, Messias-Reason IJ (2013) Association of L-ficolin levels and FCN2 genotypes with chronic Chagas disease. PLoS One 8: e60237. doi: 10.1371/journal.pone.0060237 PMID: 23593180

29. Ohtani K, Suzuki Y, Wakamiya N (2012) Biological functions of the novel collectins CL-L1, CL-K1, and CL-P1. Journal of biomedicine & biotechnology 2012/05/10: 493945. Research Support, Non-U.S. Gov't Review.

30. Hansen S, Selman L, Palaniyar N, Ziegler K, Brandt J, Kliem A, Jonasson M, Skjoedt MO, Nielsen O, Hartshorn K, Jorgensen TJ, Skjodt K, Holmskov U (2010) Collectin 11 (CL-11, CL-K1) is a MASP-1/3-associated plasma collectin with microbial-binding activity. Journal of immunology 2010/10/20: 6096–6104. Research Support, Non-U.S. Gov't.

31. Motomura W, Yoshizaki T, Ohtani K, Okumura T, Fukuda M, Fukuzawa J, Mori K, Jang SJ, Nomura N, Yoshida I, Suzuki Y, Kohgo Y, Wakamiya N (2008) Immunolocalization of a novel collectin CL-K1 in murine tissues. The journal of histochemistry and cytochemistry: official journal of the Histochemistry Society 2007/11/28: 243–252. Research Support, Non-U.S. Gov't.

32. Rooryck C, Diaz-Font A, Osborn DP, Chabchoub E, Hernandez-Hernandez V, Shamseldin H, Kenny J, Waters A, Jenkins D, Kaissi AA, Leal GF, Dallapiccola B, Carnevale F, Bitner-Glindzicz M, Lees M, Hennekam R, Stanier P, Burns AJ, Peeters H, Alkuraya FS, Beales PL (2011) Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. Nature genetics 2011/01/25: 197–203. Research Support, Non-U.S. Gov't.

33. Chang JC, Kuo HC, Hsu TY, Ou CY, Liu CA, Chuang H, Liang HM, Huang HW, Yang KD (2013) Different genetic associations of the IgE production among fetus, infancy and childhood. PloS one 2013/08/13: e70362. Comparative Study Research Support, Non-U.S. Gov't.

34. Henriksen ML, Brandt J, Andrieu JP, Nielsen C, Jensen PH, Holmskov U, Jorgensen TJ, Palarasah Y, Thielens NM, Hansen S (2013) Heteromeric complexes of native collectin kidney 1 and collectin liver 1 are found in the circulation with MASPs and activate the complement system. Journal of immunology 2013/11/01: 6117–6127. Research Support, Non-U.S. Gov't.

35. Selman L, Hansen S (2012) Structure and function of collectin liver 1 (CL-L1) and collectin 11 (CL-11, CL-K1). Immunobiology 2012/04/06: 851–863. Research Support, Non-U.S. Gov't Review.

36. Antony JS, Ojurongbe O, van Tong H, Ouf EA, Engleitner T, Akindele AA, Sina-Agbaje OR, Adeyeba AO, Kremsner PG, Velavan TP (2013) Mannose-binding lectin and susceptibility to schistosomiasis. The Journal of infectious diseases 2013/03/02: 1675–1683. Research Support, Non-U.S. Gov't.

37. Ouf EA, Ojurongbe O, Akindele AA, Sina-Agbaje OR, van Tong H, Adeyeba AO, Kremsner PG, Kun JF, Velavan T (2012) Ficolin-2 levels and FCN2 genetic polymorphisms as a susceptibility factor in schistosomiasis. The Journal of infectious diseases 2012/06/14: 562–570. Research Support, Non-U.S. Gov't.

38. Ojurongbe O, Sina-Agbaje OR, Busari A, Okorie PN, Ojurongbe TA, Akindele AA (2014) Efficacy of praziquantel in the treatment of Schistosoma haematobium infection among school-age children in

rural communities of Abeokuta, Nigeria. Infect Dis Poverty 3: 30. doi: 10.1186/2049-9957-3-30 PMID: 25215186

39. Deng J, Gold D, LoVerde PT, Fishelson Z (2003) Inhibition of the complement membrane attack complex by Schistosoma mansoni paramyosin. Infection and immunity 2003/10/24: 6402–6410. Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.

40. Cestari I, Evans-Osses I, Schlapbach LJ, de Messias-Reason I, Ramirez MI (2013) Mechanisms of complement lectin pathway activation and resistance by trypanosomatid parasites. Molecular immunology 2012/10/16: 328–334. Research Support, Non-U.S. Gov't Review.

41. Pace CN, Scholtz JM (1998) A helix propensity scale based on experimental studies of peptides and proteins. Biophys J 75: 422–427. PMID: 9649402

42. International HapMap C (2005) A haplotype map of the human genome. Nature 2005/10/29: 1299–1320. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U. S. Gov't, P.H.S.

43. Xue Y, Zhang X, Huang N, Daly A, Gillson CJ, Macarthur DG, Yngvadottir B, Nica AC, Woodwark C, Chen Y, Conrad DF, Ayub Q, Mehdi SQ, Li P, Tyler-Smith C (2009) Population differentiation as an indicator of recent positive selection in humans: an empirical evaluation. Genetics 2009/09/10: 1065–1077. Research Support, Non-U.S. Gov't.

44. Takahashi K, Ohtani K, Larvie M, Moyo P, Chigweshe L, Van Cott EM, Wakamiya N (2014) Elevated plasma CL-K1 level is associated with a risk of developing disseminated intravascular coagulation (DIC). Journal of thrombosis and thrombolysis 2014/01/30.

45. Degn SE, Jensenius JC, Thiel S (2011) Disease-causing mutations in genes of the complement system. American journal of human genetics 2011/06/15: 689–705. Research Support, Non-U.S. Gov't Review.

Cytokine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Correlation of Interleukin-6 levels and lectins during *Schistosoma haematobium* infection

Justin S. Antony [a], Olusola Ojurongbe [a,b], Christian G. Meyer [a,c], Kumarasamy Thangaraj [d], Anshuman Mishra [d], Peter G. Kremsner [a], Thirumalaisamy P. Velavan [a,e,*]

[a] *Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany*
[b] *Ladoke Akintola University of Technology, Ogbomoso, Nigeria*
[c] *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*
[d] *CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India*
[e] *Fondation Congolaise pour la Recherche Medicale, Brazzaville, People's Republic of Congo*

ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form 28 April 2015
Accepted 29 April 2015
Available online xxxx

Keywords:
IL-6
MBL
Ficolin-2
CL-K1
Schistosomiasis

ABSTRACT

Urogenital schistosomiasis caused by *Schistosoma haematobium* induces a Th2 immune response, including expression of Interleukin-6. IL-6 confers protection from experimental *Schistosoma*-induced pulmonary hypertension and modulates production of mannose-binding lectin (MBL) and other lectins. We studied IL-6 levels in schistosomiasis and its effect on lectins production. Elevated IL-6 levels occurred in cases, compared to controls. IL-6 correlated with the lectins MBL, ficolin-2 and Collectin Kidney-1 (CL-K1) in cases, but correlated inversely in controls. The study shows that IL-6 levels are elevated in individuals infected with urogenital schistosomiasis. IL-6 was also found to be correlated with the production of lectins in *S. haematobium* infection. A similar correlation between IL-6 and MBL was observed during visceral leishmaniasis.

1. Introduction

Schistosomiasis is a chronic parasitic disease due to infection with different species of trematode worms of the genus *Schistosoma*. Infection with *Schistosoma haematobium* is the main cause of urogenital schistosomiasis. The clinical presentation includes hematuria, hydronephrosis, anemia and predisposition to HIV and other infections of the urogenital tract. Schistosomiasis accounts for more than 70 million disability adjusted life years (DALYs) lost and with more than 240 million individuals currently infected it is the second most common devastating parasitic disease after malaria in sub-Saharan Africa [1].

Schistosomes induce strong Th2 immune response during infection by down-regulation of the Th1 immune response through increased production of Interleukin-6 (IL-6) [2]. IL-6 is an immunoregulatory cytokine [3], secreted by T cells and macrophages to stimulate non-specific immune responses, including fever - and acute phase responses in parasitic diseases [4]. The larvae of schistosomes are recognized by host macrophages, which induce the secretion of IL-6 [5]. Subsequently, IL-6 stimulates the expression of acute phase proteins in the liver by binding to IL-6-responsive elements of the promoter of respective genes [6]. IL6 has been found to be involved in protection against *Schistosoma*-induced pulmonary hypertension in an animal model [7].

Mannose-binding Lectin (MBL) is an acute-phase plasma protein (APP) with circulating serum levels increasing 1.5–3.0-fold during distinct infections [8]. MBL is an initiation molecule of the complement-lectin pathway and MBL deficiency is associated with a variety of diseases [9]. In the course of many infectious diseases, MBL modulates IL-6 production at the mRNA and protein level by signaling to human peripheral blood mononuclear cells to suppress the production of the pro-inflammatory cytokines IL-1α and IL-1β, and enhances the secretion of anti-inflammatory cytokines, including IL-6 [10]. Several type 1 and type 2 IL-6-responsive sequences observed in the human *MBL2* promoter region point to possible interactions of IL-6 and MBL in the pathogenesis of many disease [11].

We have previously shown that MBL, ficolin-2 and CL-K1 deficiency constitutes a risk factor for urogenital schistosomiasis [12–14]. Based on the rationale that IL-6 is involved in the pathogenesis of schistosomiasis as well as in the induction of MBL [11], we aimed to explore the role of IL-6 and its relation with MBL

* Corresponding author at: Institute of Tropical Medicine, University of Tübingen, Wilhelmstrasse 27, 72074 Tübingen, Germany. Tel.: +49 7071 2985981; fax: +49 7071 294684.
E-mail address: velavan@medizin.uni-tuebingen.de (T.P. Velavan).

http://dx.doi.org/10.1016/j.cyto.2015.04.019
1043-4666/

during *S. haematobium* infection. As ficolin-2 and CL-K1 share similarities in both their structure and function with MBL [15], we studied the correlation of IL-6 in the expression of these proteins during urogenital schistosomiasis. We also investigated the correlation of IL-6 with lectins to visceral leishmaniasis (VL).

2. Materials and methods

2.1. Study design

The study included a total of 359 individuals from the two villages Ilewo Orile, Ogun State and Ore, Osun State, southwest Nigeria. The area is highly endemic for *S. haematobium* infections. The study was designed as a cross-sectional study with individuals recruited blindly, irrespective of their infection status. Ten ml of urine were collected and screened for schistosomal eggs using a filtration technique and subsequent microscopic examination. In addition, five ml of whole venous blood were collected from all individuals for the serological assessment of anti-*Schistosoma* total IgG as an exposure marker using *Schistosoma* adult worm antigen. For individuals negative for eggs of *S. haematobium* at the first examination, urine samples were collected on two successive days to avoid biases in the fluctuation of egg shedding. Based on results of the microscopic examination of urine filtrates and the anti-*Schistosoma* total IgG antibodies tests, the study participants were assigned to three groups. Individuals of the case group were positive for *S. haematobium* eggs in urine [SEP; $n = 167$, 93 (56%) males, 74 (44%) females ($P = 0.048$); age range 2–70 years, mean egg count per 10 ml urine 1595 eggs (20-27000)]. Participants of the control subgroup 1 were negative for *S. haematobium* eggs in urine, but positive for anti-*Schistosoma* total IgG antibodies [SELP $n = 123$, 61 (50%) males, 62 (50%) females ($P = 1.0$); age range 4–80 years]. The control group 2 consisted of individuals negative for both *S. haematobium* eggs in urine and anti-*Schistosoma* total IgG antibodies [SELN $n = 69$, 43 (62%) males, 26 (38%) females ($P = 0.006$); age range 4–70]. Cases were treated with a single dose of 40 mg/kg of praziquantel. Stool samples were collected from all participants and processed using the Kato-Katz method in order to exclude *Schistosoma mansoni* infections. Cases with other infections including HIV were excluded from the study. The Ethical Committee of the Ministry of Health, Abeokuta Ogun State, Nigeria approved the study design. Informed consent in the local language Yoruba was obtained from all participants. For children, consent was sought for from their parents and/or guardians.

2.2. Measurement of IL-6

IL-6 serum levels were measured in 1:4 diluted sera of urinary schistosomiasis cases ($n = 167$) and controls ($n = 192$) using the commercially available Human IL-6 High Sensitivity ELISA (eBiosciences, San Diego, USA) following the manufacturer's instructions. The detection limit of the assay was 0.08 pg/mL. We used our previously reported serum level determination of MBL, ficolin-2 and CL-K1 [12–14] of the same samples. IL-6 levels were assessed by calculating unknown values from standard curves using the Michaelis–Menten model in the Graphpad Prism v.6.0d software (www.graphpad.com).

2.3. Statistical analysis

Kruskal–Wallis rank sum tests were applied to analyze differences of serum IL-6 levels between the study subgroups. Multivariate regression analysis was performed to adjust the confounding effects of age and gender in IL-6 serum levels (Intercooled STATA, STATA Corp., College Station, TX, USA). The correlation analyses of IL-6 were performed with MBL, ficolin-2 and CL-K1 by non-parametric Spearmańs rank coefficient tested as implemented in Graphpad Prism v.6.0d.

3. Results

IL-6 serum levels among combined (SELP + SELN) controls varied from 0.2 to 18.9 pg/ml with a median of 1.8 pg/mL. IL-6 serum levels were significantly elevated in *S. haematobium* egg-positive (SEP) cases (mean 8.7 pg/ml) when compared to the both control subgroups separated or merged. (SELP: mean 4.9 pg/ml, SELN: mean 5.9 pg/ml, SELP + SELN: mean 5.3 pg/ml) ($P < 0.0001$; Fig. 1A). The gender distribution was observed to be significant, with more male individuals in both SEP and SELN subgroups. This was a cross sectional study and both male and female individuals were recruited blind irrespective of their prior infection status, hence this observation was likely. However, multivariate analysis of gender and also age with IL-6 did not reveal any relevant contribution of these confounders to circulating IL-6 serum levels. We further correlated IL-6 serum levels with those of the previously reported lectins (MBL, ficolin-2, CL-K1) in both SEP cases and control subgroups. IL-6 serum levels were positively correlated with MBL (Spearman's rho coefficient: $\rho = 0.189$, $P = 0.01$), ficolin-2 ($\rho = 0.192$, $P = 0.01$) and CL-K1 ($\rho = 0.292$, $P = 0.0002$) in cases (Fig. 1B). Notably, IL-6 was inversely correlated with MBL ($\rho = -0.239$, $P = 0.001$) and ficolin-2 ($\rho = -0.239$, $P = 0.001$) in the pooled control subgroups (Fig. 1C). CL-K1 did not show any significant correlation with IL-6 in controls. We observed a similar correlation when the control subgroups were analyzed separately (data not shown).

In order to verify the association of IL-6 with lectins production in other parasitic disease, we measured the IL-6 levels in the sera from Indian VL cases ($n = 58$) and controls ($n = 30$) using the same ELISA kit and correlated with lectins MBL, ficolin-2 levels. Detailed information on the study group and the study design is provided elsewhere [16]. We observed that IL-6 levels were elevated in VL cases (mean 7.07 pg/mL) compared to controls (mean 3.1 pg/mL) ($P = 0.003$; Fig. 2A). In VL cases, the MBL levels were positively correlated with IL-6 (Spearman's rho coefficient: $\rho = 0.3$, $P = 0.01$; Fig. 2B) while ficolin-2 did not show any significant correlation with IL-6. No correlation was observed between IL-6 and lectins in control subjects (Fig. 2C).

4. Discussion

The pathogenesis of urogenital schistosomiasis is complex [17]. Cytokines are released in response to infection and they are, directly or indirectly, involved in many pathophysiological events [18]. Here, we studied the role of IL-6 in urogenital schistosomiasis. Our findings indicate that IL-6 serum levels were higher among *S. haematobium* egg-positive individuals compared to the *S. haematobium* sero-positive and sero-negative control subgroups. This is in line with reports where IL-6 was observed to be elevated in both natural and experimental *Schistosoma* infection [2,19,20]. IL-6 serum levels have previously been shown to be also elevated in patients with bladder cancer associated with *S. haematobium* infection, correlating with advanced tumor grade and exhibiting an independent functional role as a disease modifier [21]. Our findings emphasize the potential role of IL-6 in the pathogenesis of urogenital schistosomiasis. Likewise, IL-6 levels were elevated in investigated VL cases compared to controls suggesting the role of this cytokine in VL pathogenesis [22].

In the present study we hypothesized that IL-6 might exert synergistic effects with MBL and other lectins during *S. haematobium* infection [10]. We observed a positive correlation of the lectins MBL, ficolin-2 and CL-K1 with IL-6 in urogenital schistosomiasis.

Please cite this article in press as: Antony JS et al. Correlation of Interleukin-6 levels and lectins during *Schistosoma haematobium* infection. Cytokine (2015), http://dx.doi.org/10.1016/j.cyto.2015.04.019

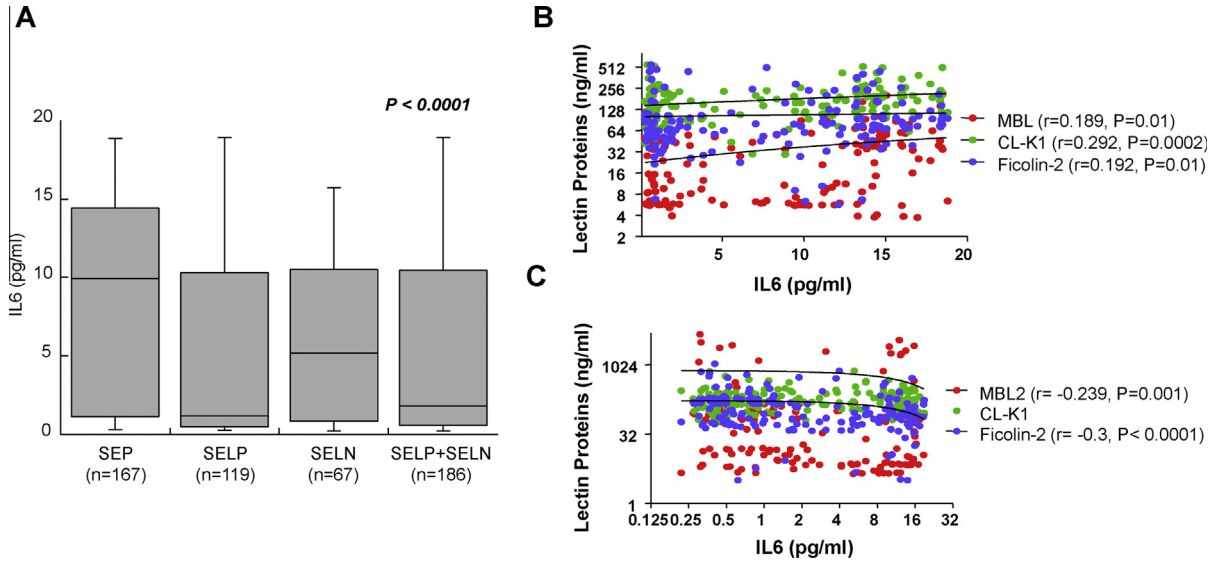

Fig. 1. (A) Distribution of IL-6 serum levels in individuals positive for *Schistosoma haematobium* eggs in urine (SEP cases), individuals negative for *S. haematobium* eggs in urine, but total IgG positive (adult *S. haematobium* antigen; SELP control subgroup 1) and individuals negative for both *S. haematobium* eggs in urine and negative for anti-*Schistosoma* antibodies (SELN control subgroup 2) along with the combined control subgroups. Box-plots illustrate medians with 25 and 75 percentiles with whiskers to 10 and 90 percentiles. $P < 0.0001$ illustrated in the figure is calculated by Kruskal–Wallis rank sum test. (B) Correlation analysis of IL-6 serum levels with MBL, ficolin-2 and CL-K1 in cases, (C) combined control subgroups. For proper scaling ficolin-2 serum values, these were divided by the factor of 10.

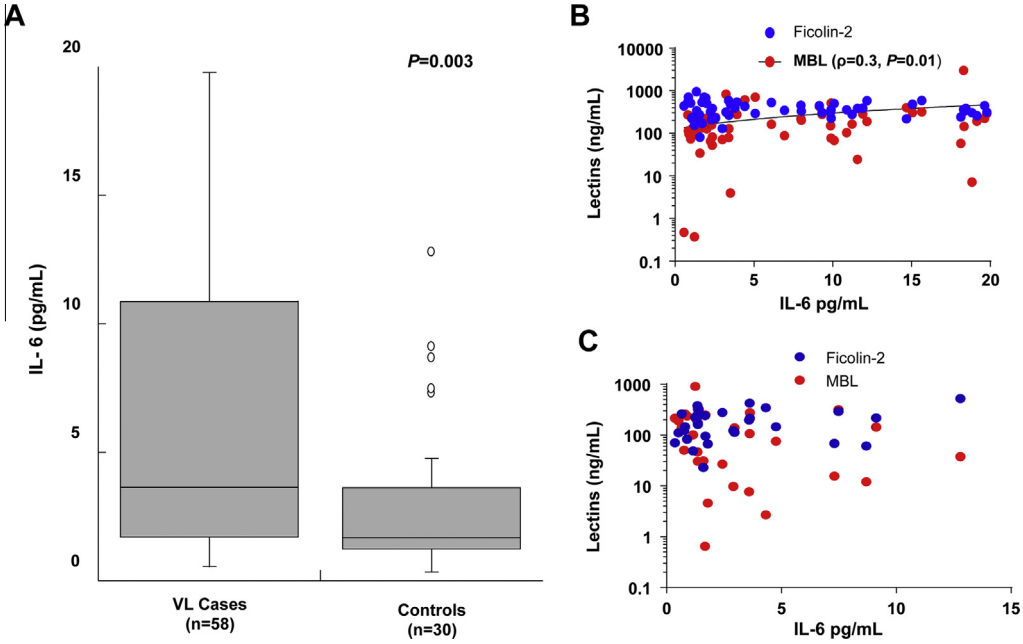

Fig. 2. Distribution of IL-6 serum levels in visceral leishmaniasis cases and healthy controls. Box-plots illustrate medians with 25 and 75 percentiles with whiskers to 10 and 90 percentiles. $P = 0.003$ illustrated in the figure is calculated by Wilcoxon–Mann–Whitney rank sum test. Numbers in parenthesis represent the number of samples (A). Correlation analysis of IL-6 serum levels with MBL and ficolin-2 in VL cases (B) and controls (C). For proper scaling ficolin-2 serum values, these were divided by the factor of 10.

Similar observations of interaction of IL-6 and the C-reactive protein (CRP) were made in HIV patients with the immune reconstitution inflammatory syndrome (IRIS), where IL-6 and CRP levels were positively correlated [23]. It is clear that MBL and other functional analogues may be modulated by IL-6 stimuli. TNF-α, IL-1, and IL-6 essentially regulate the acute phase response proteins (APP), including MBL, which responds to IL-6 and glucocorticoids [24] by up-regulating the APP genes. However, in spite of the up-regulation by IL-6 of APP genes, our observations indicate that *S. haematobium* parasite factors contribute to lower circulating serum levels of MBL, ficolin-2 and CL-K1 by consumption of complement [12–14,25,26]. Corresponding observations of MBL and IL-6 have been described in meningococcal infection [27]. A similar correlation between IL-6 and MBL was observed in VL while no association between ficolin-2 and IL-6 was observed. A larger sample size and functional studies may help to better understand the contribution of IL-6 with other investigated lectins such as ficolin-2.

Schistosomiasis is a strong inducer of a Th2 immune response with IL-6 expression. During the infection, IL-6 enhances IL-10

Please cite this article in press as: Antony JS et al. Correlation of Interleukin-6 levels and lectins during *Schistosoma haematobium* infection. Cytokine (2015), http://dx.doi.org/10.1016/j.cyto.2015.04.019

production and inhibits IL-12 and IFN-γ, thus directing immunity towards a Th2 response through negative regulation of the type 1 response [2]. MBL serum levels were observed to be inversely correlated with IL-6 and CRP levels in a cystic fibrosis patient who underwent partially successful MBL therapy [28]. In that case, treatment with MBL could modulate the inflammatory reaction regulated by IL-6. IL-6 was also observed to interact with monocytes and platelets in killing of *S. mansoni* parasites.

Notably, we observed an inverse correlation of IL-6 with MBL and ficolin-2, but not with CL-K1 serum levels, both in seropositive and seronegative controls. As healthy individuals are not exposed to inflammatory stimuli, the negative correlation of IL-6 levels with the lectins MBL and ficolin-2 may be explained [29]. However, the exact mechanism responsible for the inverse correlation of MBL and ficolin-2 with IL-6 and the absence of any correlation of IL-6 with CL-K1 in healthy individuals needs to be explored further.

5. Conclusion

This study aimed at exploring the role of IL-6 in urogenital schistosomiasis caused by *S. haematobium*. We have shown that IL-6 levels are elevated in infected individuals and that IL-6 levels correlate with the production of lectins during the schistosomiasis. We observed similar correlation of IL-6 with lectin MBL during VL. These findings warrant further investigation of other Th2 specific cytokines and their roles in schistosomiasis or other diseases in general and the lectin pathway in particular.

Acknowledgements

We thank Abass Busari, Olaolu Abdulazeez, Akeem Akindele and Olawumi Sina-Agbaje for their support. We also thank the staff of the Schistosomiasis Control Unit, Ministry of Health, Abeokuta, and the community members for their participation in the study. This study was funded by an internal institutional grant to TPV. Olusola Ojurongbe was supported by the Alexander von Humboldt Foundation.

References

[1] Rinaldi G, Young ND, Honeycutt JD, Brindley PJ, Gasser RB, Hsieh MH. New research tools for urogenital schistosomiasis. J Infect Dis 2014:jiu527. http://dx.doi.org/10.1093/infdis/jiu527.

[2] La Flamme AC, MacDonald AS, Pearce EJ. Role of IL-6 in directing the initial immune response to schistosome eggs. J Immunol 2000;164:2419–26.

[3] Boulanger MJ, Chow DC, Brevnova EE, Garcia KC. Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex. Science 2003;300:2101–4. http://dx.doi.org/10.1126/science.1083901. 300/5628/2101.

[4] Bode JG, Albrecht U, Haussinger D, Heinrich PC, Schaper F. Hepatic acute phase proteins–regulation by IL-6- and IL-1-type cytokines involving STAT3 and its crosstalk with NF-kappa B-dependent signaling. Eur J Cell Biol 2012;91:496–505. http://dx.doi.org/10.1016/j.ejcb.2011.09.008. S0171-9335(11)00180-4.

[5] Jenkins SJ, Hewitson JP, Ferret-Bernard S, Mountford AP. Schistosome larvae stimulate macrophage cytokine production through TLR4-dependent and -independent pathways. Int Immunol 2005;17:1409–18. http://dx.doi.org/10.1093/intimm/dxh319. dxh319.

[6] Dalmon J, Laurent M, Courtois G. The human beta fibrinogen promoter contains a hepatocyte nuclear factor 1-dependent interleukin-6-responsive element. Mol Cell Biol 1993;13:1183–93.

[7] Graham BB, Chabon J, Kumar R, Kolosionek E, Gebreab L, Debella E, et al. Protective role of IL-6 in vascular remodeling in Schistosoma pulmonary hypertension. Am J Respir Cell Mol Biol 2013;49:951–9. http://dx.doi.org/10.1165/rcmb.2012-0532OC.

[8] Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. Clin Exp Immunol 1992;90:31–5.

[9] Degn SE, Jensenius JC, Thiel S. Disease-causing mutations in genes of the complement system. Am J Hum Genet 2011;88:689–705. http://dx.doi.org/10.1016/j.ajhg.2011.05.011. S0002-9297(11)00204-7 [pii].

[10] Fraser DA, Bohlson SS, Jasinskiene N, Rawal N, Palmarini G, Ruiz S, et al. C1q and MBL, components of the innate immune system, influence monocyte cytokine expression. J Leukoc Biol 2006;80:107–16. http://dx.doi.org/10.1189/jlb.1105683.

[11] Naito H, Ikeda A, Hasegawa K, Oka S, Uemura K, Kawasaki N, et al. Characterization of human serum mannan-binding protein promoter. J Biochem 1999;126:1004–12.

[12] Antony JS, Ojurongbe O, van Tong H, Ouf EA, Engleitner T, Akindele AA, et al. Mannose-binding lectin and susceptibility to schistosomiasis. J Infect Dis 2013;207:1675–83. http://dx.doi.org/10.1093/infdis/jit081.

[13] Ouf EA, Ojurongbe O, Akindele AA, Sina-Agbaje OR, van Tong H, Adeyeba AO, et al. Ficolin-2 levels and FCN2 genetic polymorphisms as a susceptibility factor in schistosomiasis. J Infect Dis 2012;206:562–70. http://dx.doi.org/10.1093/infdis/jis396.

[14] Antony JS, Ojurongbe O, Kremsner PG, Velavan TP. Lectin complement protein Collectin 11 (CL-K1) and susceptibility to urinary schistosomiasis. PLoS Negl Trop Dis 2015;9:e0003647. http://dx.doi.org/10.1371/journal.pntd.0003647. PNTD-D-14-01460 [pii].

[15] Zhang XL, Ali MA. Ficolins: structure, function and associated diseases. Adv Exp Med Biol 2008;632:105–15.

[16] Mishra A, Jha AN, van TH, Singh VK, Gomes CE, Singh L, et al. Analysis of genetic variants in the IL4 promoter and VNTR loci in Indian patients with Visceral Leishmaniasis. Hum Immunol 2014;75:1177–81. http://dx.doi.org/10.1016/j.humimm.2014.10.007. S0198-8859(14)00472-8 [pii].

[17] Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet 2006;368:1106–18. http://dx.doi.org/10.1016/S0140-6736(06)69440-3. S0140-6736(06)69440-3 [pii].

[18] Hopkins SJ. The pathophysiological role of cytokines. Leg Med (Tokyo) 2003;5(Suppl 1):S45–57. S1344622302000883 [pii].

[19] McDonald EA, Pond-Tor S, Jarilla B, Sagliba MJ, Gonzal A, Amoylen AJ, et al. Schistosomiasis japonica during pregnancy is associated with elevated endotoxin levels in maternal and placental compartments. J Infect Dis 2014;209:468–72. http://dx.doi.org/10.1093/infdis/jit446.

[20] Khalil RM, Hultner L, Mailhammer R, Luz A, Moeller J, Mohamed AA, et al. Kinetics of interleukin-6 production after experimental infection of mice with Schistosoma mansoni. Immunology 1996;89:256–61.

[21] El-Salahy EM. Evaluation of cytokeratin-19 & cytokeratin-20 and interleukin-6 in Egyptian bladder cancer patients. Clin Biochem 2002;35:607–13.

[22] Costa DL, Rocha RL, Carvalho RM, Lima-Neto AS, Harhay MO, Costa CH, et al. Serum cytokines associated with severity and complications of kala-azar. Pathog Glob Health 2013;107:78–87. http://dx.doi.org/10.1179/2047773213Y.0000000078.

[23] Barber DL, Andrade BB, McBerry C, Sereti I, Sher A. Role of IL-6 in Mycobacterium avium–associated immune reconstitution inflammatory syndrome. J Immunol 2014;192:676–82. http://dx.doi.org/10.4049/jimmunol.1301004.

[24] Duan HO, Simpson-Haidaris PJ. Functional analysis of interleukin 6 response elements (IL-6REs) on the human gamma-fibrinogen promoter: binding of hepatic Stat3 correlates negatively with transactivation potential of type II IL-6REs. J Biol Chem 2003;278:41270–81. http://dx.doi.org/10.1074/jbc.M304210200.

[25] Klabunde J, Berger J, Jensenius JC, Klinkert MQ, Zelck UE, Kremsner PG, et al. Schistosoma mansoni: adhesion of mannan-binding lectin to surface glycoproteins of cercariae and adult worms. Exp Parasitol 2000;95:231–9. http://dx.doi.org/10.1006/expr.2000.4539. S0014-4894(00)94539-9.

[26] Marikovsky M, Arnon R, Fishelson Z. Proteases secreted by transforming schistosomula of Schistosoma mansoni promote resistance to killing by complement. J Immunol 1988;141:273–8.

[27] Jack DL, Read RC, Tenner AJ, Frosch M, Turner MW, Klein NJ. Mannose-binding lectin regulates the inflammatory response of human professional phagocytes to Neisseria meningitidis serogroup B. J Infect Dis 2001;184:1152–62. http://dx.doi.org/10.1086/323803.

[28] Garred P, Pressler T, Lanng S, Madsen HO, Moser C, Laursen I, et al. Mannose-binding lectin (MBL) therapy in an MBL-deficient patient with severe cystic fibrosis lung disease. Pediatr Pulmonol 2002;33:201–7.

[29] Czarkowska-Paczek B, Bartlomiejczyk I, Gabrys T, Przybylski J, Nowak M, Paczek L. Lack of relationship between interleukin-6 and CRP levels in healthy male athletes. Immunol Lett 2005;99:136–40. http://dx.doi.org/10.1016/j.imlet.2005.02.006. S0165-2478(05)00032-5 [pii].

Please cite this article in press as: Antony JS et al. Correlation of Interleukin-6 levels and lectins during *Schistosoma haematobium* infection. Cytokine (2015), http://dx.doi.org/10.1016/j.cyto.2015.04.019

PLOS | ONE

RESEARCH ARTICLE

Association of Ficolin-2 Serum Levels and *FCN2* Genetic Variants with Indian Visceral Leishmaniasis

Anshuman Mishra[1☯], Justin S. Antony[2☯], Pandarisamy Sundaravadivel[1], Hoang Van Tong[2], Christian G. Meyer[2], Reshma D. Jalli[1], Thirumalaisamy P. Velavan[2,3‡*], Kumarasamy Thangaraj[1‡]

1 Council of Scientific and Industrial Research—Centre for Cellular and Molecular Biology, Hyderabad, India, **2** Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany, **3** Fondation Congolaise pour la Recherche Medicale, Brazzaville, Republic of Congo

☯ These authors contributed equally to this work.
‡ These authors are shared last authors on this work.
* velavan@medizin.uni-tuebingen.de

Citation: Mishra A, Antony JS, Sundaravadivel P, Tong HV, Meyer CG, Jalli RD, et al. (2015) Association of Ficolin-2 Serum Levels and *FCN2* Genetic Variants with Indian Visceral Leishmaniasis. PLoS ONE 10(5): e0125940. doi:10.1371/journal.pone.0125940

Academic Editor: James D. Chalmers, University of Dundee, UNITED KINGDOM

Received: February 5, 2015

Accepted: March 26, 2015

Published: May 12, 2015

Data Availability Statement: All relevant data are within the paper.

Funding: Anshuman Mishra was supported by Department of Biotechnology (www.dbtindia.nic.in), Research Associate Programme, Government of India. Kumarasamy Thangaraj was supported by Council for Scientific and Industrial Research (www.csir.res.in) Network project (Genesis-BSC0121) and Biotechnology and Biological Sciences Research Council (http://www.bbsrc.com), United Kingdom (BB/H009337). The authors acknowledge the support by the Deutsche Forschungsgemeinschaft (DFG) and

Abstract

Background

Visceral leishmaniasis (VL), one of the neglected tropical diseases, is endemic in the Indian subcontinent. Ficolins are circulating serum proteins of the lectin complement system and involved in innate immunity.

Methods

We have estimated ficolin-2 serum levels and analyzed the functional variants of the encoding gene *FCN2* in 218 cases of VL and in 225 controls from an endemic region of India.

Results

Elevated levels of serum ficolin-2 were observed in VL cases compared to the controls (adjusted $P<0.0001$). The genetic analysis revealed that the *FCN2*structural variant +6359 C>T (p.T236M) was associated with VL (OR=2.2, 95% CI=1.23-7.25, $P=0.008$) and with high ficolin-2 serum levels. We also found that the *FCN2*AAAC* haplotype occurred more frequently among healthy controls when compared to cases (OR=0.59, 95%CI=0.37-0.94, $P=0.023$).

Conclusions

Our findings indicate that the *FCN2* variant +6359C>T is associated with the occurrence of VL and that ficolin-2 serum levels are elevated in *Leishmania* infections.

Open Access Publishing Fund of Tuebingen University. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

Visceral leishmaniasis (VL; Kala-Azar), a neglected tropical disease strongly associated with poverty, claims 400.000 new cases and 40.000 deaths annually [1]. VL leads to a loss of about 2 million disability adjusted life years (DALYs) every year [2]. The vector-borne infection occurs in the four distinct clinical manifestations as cutaneous leishmaniasis, muco-cutaneous leishmaniasis, VL and post-kala-azar dermal leishmaniasis [3]. VL is the severest form and severely affects visceral organs including the spleen, liver and lymph nodes [4]. Although transmission of VL has been reported in 66 countries, more than 90% of the disease burden are observed in six countries only, viz. Bangladesh, India, Nepal, Sudan, Ethiopia and Brazil [3]. Among these countries, the Indian sub-continent (India, Nepal and Bangladesh) harbours 67% of the global VL disease burden [5]. In particular, the Bihar state of India shares 50% of VL and is considered a "hot spot" of VL [6]. Inadequate vector control practice and disease management have been claimed to be responsible for the increased incidence of VL and associated mortality in India [7].

Leishmania donovani is the causative agent of VL in India. The organism is transmitted to mammalian hosts by infective bites of the sandfly *Phlebotomus argentipes*. *L. donovani* is a unicellular trypanosomatid protozoan parasite with a dimorphic life cycle between the sandfly vector (extracellular promastigotes) and the human host (intracellular amastigotes) [8]. Both developmental stages of *L. donovani* are coated with various secreted and membrane bound phosphoglycans. During the promastigote stage, abundant lipohosphoglycan (LPG) and gp63 are expressed, which aid immune evasion of the parasite by inhibiting the phagolysosome biogenesis in phagocytes [9]. Further, these glycoconjugates facilitate the parasite´s survival in the hostile macrophage environment [10]. However, LPG and gp36 may also serve as pathogen-associated molecular patterns (PAMPs) which are recognized by pattern recognition molecules (PRMs) of the innate system such as complement serum proteins, mannose-binding lectin (MBL), ficolins (FCN), other soluble C-type lectins and toll-like receptors [11]. Serum complement activating pattern recognition molecules act in a first-line innate defense against promastigotes inoculated by the sandfly bite. *Leishmania* parasites have developed various evasion strategies to avoid the lytic action of the complement system. The parasites use host complement proteins to escape the immune attack by entering into macrophages [12]. Mannose-binding lectin (MBL), a circulating serum protein, recognizes the carbohydrate domain of *L. major*, *L. mexicana*, and *L. braziliensis*. MBL binds to the surface of *Leishmania* promastigotes to opsonize the parasites. Upon binding to parasites, MBL initiates the complement cascade and provides an additional uptake mechanism of parasites by enhancing opsonophagocytosis and protects them from the immune attack [13,14] and, thus, modulates the clinical outcome of VL [15].

Ficolins are serum complement lectins that are structurally and functionally analogous to MBL [16] and, hence, expected to modify the clinical outcome of VL due to their involvement in innate immunity. Interestingly, a significant association of a distinct *FCN2* haplotype with cutaneous leishmaniasis has been reported from a Syrian population [17]. Ficolins are a group of complement activating pattern recognition molecules consisting of a collagen-like tail region and a fibrinogen-like domain (FBG) [18]. Three types of ficolins (Ficolin-1, -2, -3) of similar structure exist in humans. These types have differential tissue expression patterns and functions [19]. The role of ficolin-2, as an innate immunity component, has been studied in several infectious diseases including Hepatitis B, schistosomiasis, Chagas disease and others [16,20–22]. Ficolin-2 recognizes superficial acetylated compounds of invading pathogens by their FBG domain and initiates the lectin complement cascade [23]. The *FCN2* gene localizes to chromosome 9q34.3 (OMIM 601624) and hepatic cells predominantly express the corresponding

protein. The variants in the promoter region of *FCN2* gene at positions -986A>G, -602G>A and -4A>G have been observed to modulate the circulating ficolin-2 concentration in a dose-dependent manner. The non-synonymous exon-8 variant alleles at positions +6359C>T and +6424G>T were shown to exhibit differential binding affinities to acetylated compounds when compared to the wildtype reference alleles [24]. Studies have shown that inter-individual variation of circulating ficolin-2 concentration are correlated with polymorphisms in the promoter and exon-8 regions [25].

Although it has been showed that *FCN2* gene polymorphisms and haplotypes are associated with cutaneous leishmaniasis [17], no investigations of ficolins have so far focused on VL. Moreover, we recently observed that functional *MBL2* polymorphisms and lower MBL levels confer relative protection against VL (unpublished). As ficolin-2 shares similarities both in structure and function with MBL [26], we aimed to explore the role of potentially important *FCN2* gene variants and circulating ficolin-2 levels in VL in our Indian study group. Three promoter SNPs (-986A>G, -602G>A and -4A>G) and two structural SNPs in exon 8 (+6359C>T and +6424G>T) were genotyped and studied.

Materials and Methods

Ethics statement

Informed written consent was obtained either from the participating individual or from the parents/guardians if an individual was less than 18 years old. The study was approved by the Institutional Ethical Committee (IEC) of the CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India. Permission was also sought for and obtained from district government hospitals.

Study design and sample collection

This is a case-control study matched for ethnicity, sex and geographical location. All cases and controls were recruited through multiple field visits from villages located within a radius of ~120 km from the city of Muzaffarpur in the Bihar state of India. Previous epidemiological studies of VL have indicated that the Bihar state is a hot spot for VL with an average annual incidence of 2.49/1000 individuals [6]. The sample size was calculated prior to recruitment using the Open Epi platform (http://www.openepi.com/) based on the incidence rate and the risk of VL in the study area. A total of 443 unrelated subjects (218 cases and 225 healthy controls) were recruited. The mean age of VL cases was 28.7±16.7 and 35.3±16.2 in healthy controls ($P = 0.001$). No significant difference in the male/female ratio was observed in cases (125:95) and controls (122:93). The cases were determined based on the clinical features of VL in medical records issued by government hospitals in the study region. Typical clinical features of the cases included fever with rigors and chills and significant splenomegaly. Cases were tested with the rk39 leishmanin antigen by nitrocellulose dipstick tests (InBios International, Seattle, USA). The control subjects were free of any relevant infectious disease. Pregnant women, cases with other infections, healthy controls with a family history of VL and relatives of cases were excluded from the study. About 5.0 mls of full venous blood were collected from study subjects for serological and genetic studies. The samples were immediately transported to the lab and the serum samples were separated from whole blood and stored in the same type of tubes at -20°C until further use.

DNA isolation and *FCN2* genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the protocol described previously [27]. The reference genomic sequence was retrieved from the Ensembl database (www.ensembl.org). The five *FCN2* variants studied were PCR amplified from two genomic regions. The three promoter variants -986A>G, -602G>A and -4A>G were amplified by the primer pairs `PromF-5'-ATTGAAGGAAAATCCGATGGG-3'` and `PromR-5'-GAAGCCACC AATCACGAAG-3'`, and the two exon-8 variants +6359 C>T and +6424 G>T were amplified using the primer pairs `Exon8F-5'-CCAGCTCCCATGTCTAAAGG-3'` and `Exon8R-5'-TTACAAACCGTAGGGCCAAG-3'`. Primers were designed by Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast) and synthesized commercially (Eurofins, Bangalore, India). The target regions were amplified using an Emerald PCR master mix (TaKaRa, Shiga, Japan) and reactions were carried out in the ABI GeneAmp PCR system 9700. The thermal cycling parameters for both amplicons were: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 30 seconds and elongation at 72°C for 1 minute. PCR products were purified using Exo-SAP-IT (USB-Affymetrix, Santa Clara, USA) and 1.0 μl of the products were directly used as templates for sequencing using the BigDye terminator (v.3.1) cycle sequencing kit (Applied Biosystems, Texas, USA) on an ABI 3730XL DNA Analyzer. Variations were identified by assembling DNA sequences with the reference sequence using AutoAssembler software (Applied Biosystems, Texas, USA) and were reconfirmed visually from their electropherograms.

Ficolin-2 serological assay

Ficolin-2 levels were measured in the sera of VL cases (n = 166) and healthy controls (n = 85) using the human Ficolin-2 ELISA kit following manufacturer's instructions (Hycult Biotech, Uden, The Netherlands). The detection limit of the assay was 16 ng/mL.

Statistical analysis

Data were analyzed using the STATA software (Intercooled STATA, STATA Corp., College Station, TX, USA) and the level of significance was set to a P value of <0.05. Genotype or haplotype frequencies were calculated by simple gene counting and by expectation-maximum (EM) algorithm and the deviations from Hardy-Weinberg equilibrium were tested using the random-permutation procedure as implemented in the Arlequin v.3.5.1.2 software (http://lgb.unige.ch/arlequin). The linkage disequilibrium (LD) analysis was performed using Haploview v.3.2 (http://broadinstitute.org/haploview). Multivariate analysis was performed after adjustment with the confounding factors such as age, ethnicity and gender using the STATA software. In all comparisons, P values <0.05 were considered significant. Kruskal-Wallis or Wilcoxon-Mann-Whitney rank sum tests were applied wherever appropriate to analyze the correlation of serum ficolin-2 levels with *FCN2* variants and haplotypes by using the Kaleidagraph software (www.synergy.com).

Results

Association of *FCN2* variants with the risk of VL

The genotype and allele frequencies for the variants -986G>A, -602A>G, -4A>G and +-6359C>T) in VL cases and controls were in Hardy-Weinberg equilibrium ($P>0.05$). This did not apply to the variant +6424G>T in VL cases. This variant was excluded from further analyses. The LD patterns of the *FCN2* variants are given in Fig 1. The LD plot indicates that the promoter variants -986G>A, -4A>G and the exon 8 variant +6359C>T were in strong LD with

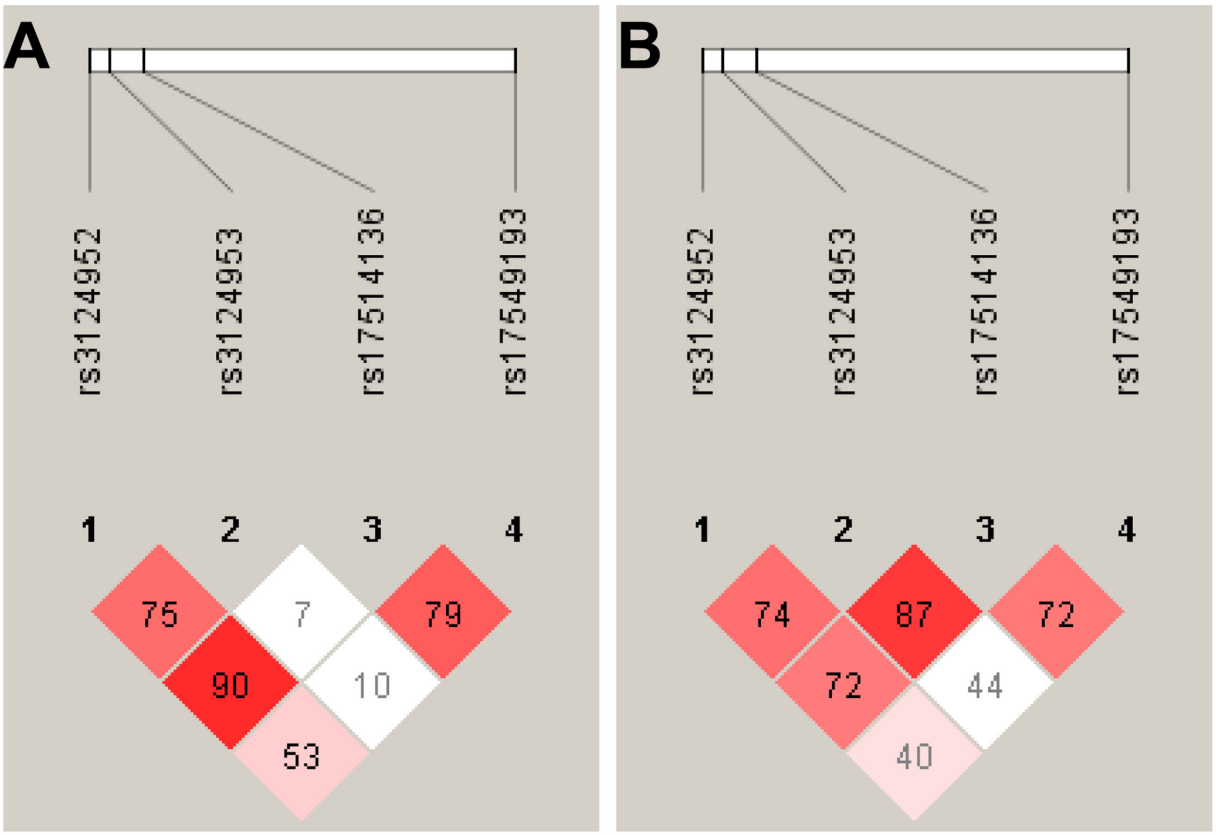

Fig 1. Linkage disequilibrium (LD) pattern of SNPs studied. (A): LD pattern of *FCN2* variants in visceral leishmaniasis cases and (B): LD pattern of *FCN2* variants in healthy controls. Numbers indicate the D' value expressed as percentile. Open squares indicate the high degree of LD (LD coefficient D' = 1) between pairs of variants. The red squares indicate pairs in strong LD with LOD scores.

doi:10.1371/journal.pone.0125940.g001

each other both in cases and controls. The variant -602A>G was in LD with +6359C>T only in controls. Significant differences were observed both in genotype and allele distributions between cases and controls for the non-synonymous variant +6359C>T (p.Thr236Met). The homozygous genotype *+6359TT* occurred more frequently among VL cases compared to controls after adjusting for age, sex and ethnicity (OR = 2.2, 95%CI = 1.23–7.25, $P = 0.008$), indicating that this variant was associated with an increased risk for *L. donovani* infection (Table 1). We observed a similar effect of the *+6359T* variant, when different genetic models are employed [Allelic: OR = 1.4, 95%CI = 1.02–1.94, $P = 0.03$; Recessive: OR = 2.2, 95%CI = 1.23–7.25, $P = 0.008$] (Table 1). The different genetic models indicate that the *+6359T* minor allele increases the susceptibility of *L. donovani* infection. The other investigated *FCN2* variants were not significantly associated with VL.

The distribution of reconstructed *FCN2* haplotypes including variants -986G>A, -602A>G, -4A>G and +6359C>T are summarized in Table 2. Fifteen secretor haplotypes were observed. The four haplotypes *FCN2*GGAC*, **AGGT*, **AAAC* and **GGAT* occurred at frequencies >10%. The reconstructed haplotype *FCN2*AAAC* was found more frequently in healthy controls compared to VL cases (OR = 0.59, 95%CI = 0.37–0.94, $P = 0.023$).

Table 1. Distribution of *FCN2* genotypes and alleles among visceral leishmaniasis cases and healthy controls.

rs17549193 (+6359C>T) (p.T236M)	VL Cases n = 204 (%)	Controls n = 223 (%)	OR (95% CI)	$P^{\#}$ value
Genotype				
CC	110 (53.9)	134 (60.1)	1	Reference
CT	72 (35.2)	80 (35.8)	NA	NS
TT	22 (10.7)	9 (4.1)	2.2 (1.23–7.25)	0.008
Allele				
C	292 (71.5)	348 (78)	1	Reference
T	116 (28.5)	98 (22)	1.4 (1.02–1.94)	0.03
Dominant				
CC	110 (53.9)	134 (60.1)	1	Reference
CT+TT	94 (46.1)	89 (39.9)	NA	NS
Recessive				
CC+CT	182 (89.3)	214 (95.9)	1	Reference
TT	22 (10.7)	9 (4.1)	2.2 (1.23–7.25)	0.008

Note: CI, confidence interval; OR, odds ratio; NS, not significant; NA, not applicable. Percentage may not add up to 100 due to rounding errors

[#] Adjusted *P* values for age, gender and ethnicity

doi:10.1371/journal.pone.0125940.t001

Table 2. Association of functional *FCN2* haplotypes and visceral leishmaniasis.

FCN2 Haplotypes (-986/-602/ -4/+6359)	VL Cases n = 408(%)	Controls n = 446(%)	OR (95% CI)	$P^{\#}$ value
GGAC	225 (55.1)	236 (52.9)	NA	NS
AGGT	54 (13.2)	53 (11.8)	NA	NS
AAAC	**36 (8.8)**	**62 (13.9)**	**0.59 (0.37–0.94)**	**0.023**
GGAT	24 (5.8)	20 (4.4)	NA	NS
AAAT	16 (3.9)	12 (2.6)	NA	NS
AGAC	14 (3.4)	24 (5.3)	NA	NS
AGGC	9 (2.2)	13 (2.9)	NA	NS
AAGT	8 (1.9)	0	NA	NA
AGAT	8 (1.9)	0	NA	NA
GAAC	7 (1.7)	10 (2.2)	NA	NS
GGGT	4 (0.9)	12 (2.6)	NA	NA
GAAT	2 (0.5)	0	NA	NA
AAGC	1 (0.2)	0	NA	NA
GGGC	0	3 (0.6)	NA	NA
GAGT	0	1 (0.2)	NA	NA

Note: CI, confidence interval; OR, odds ratio; NS, not significant; NA, not applicable. Percentage may not add up to 100 due to rounding errors

[#]Adjusted *P* values for age, gender and ethnicity

doi:10.1371/journal.pone.0125940.t002

Ficolin-2 serum levels and risk of VL

Ficolin-2 serum levels were significantly higher in VL cases (mean 2.77 μg/ml) compared to healthy controls (mean 1.94 μg/ml) (adjusted $P<0.0001$ for age, sex and ethnicity; Fig 2). Ficolin-2 levels are significantly distributed across different +6359 genotypes in controls ($P = 0.03$; Fig 3). Serum ficolin-2 levels in cases with the reconstructed *FCN2*AAAC* haplotypes were significantly higher than those measured in individuals of the control group ($P = 0.01$; Fig 4).

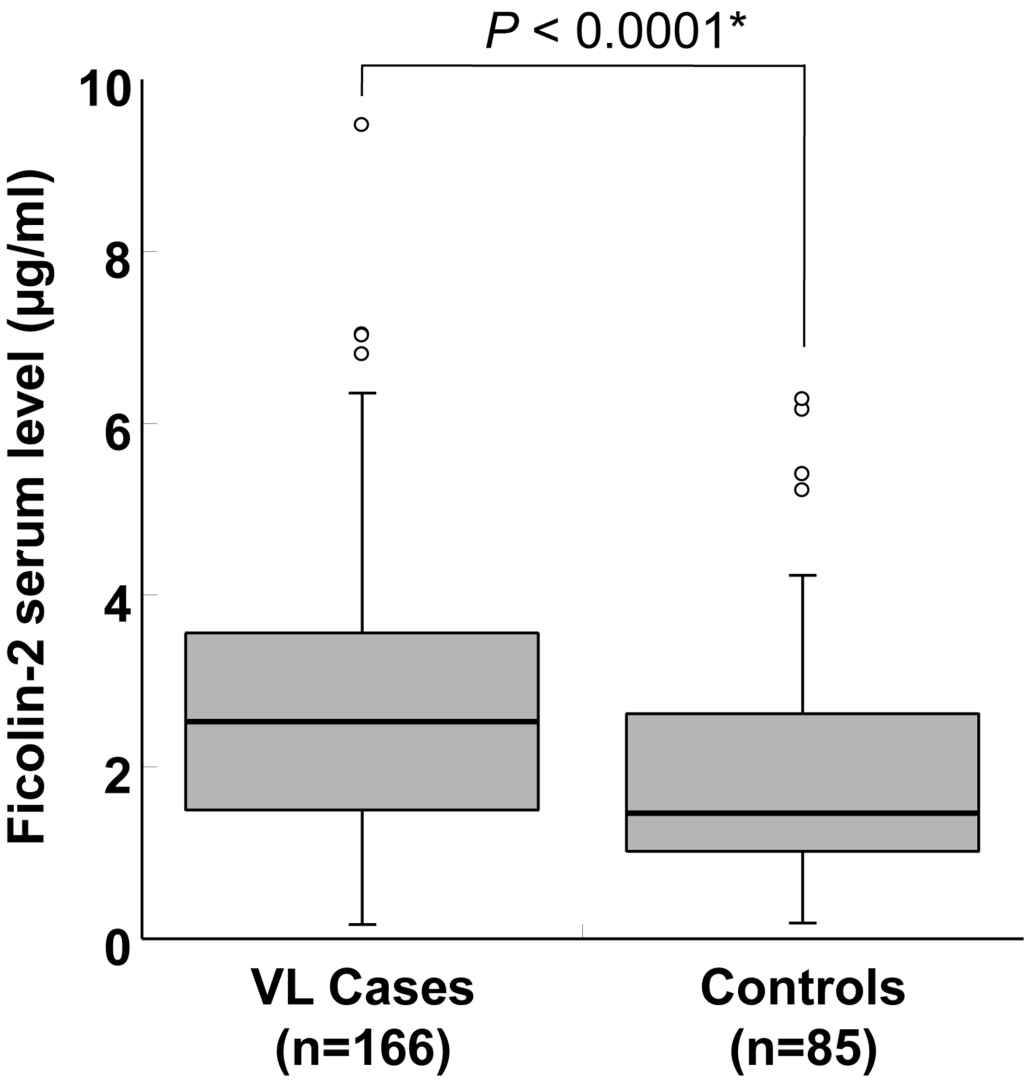

Fig 2. Distribution of ficolin-2 serum levels in visceral leishmaniasis cases and healthy controls. Box-plots illustrate medians with 25 and 75 percentiles with whiskers to 10 and 90 percentiles. **P* values were calculated by multivariate analysis adjusted for age, gender and ethnicity. Numbers in parenthesis represent the number of samples.

doi:10.1371/journal.pone.0125940.g002

Discussion

Visceral leishmaniasis develops when *L. donovani* parasites are successfully inoculated and survive the first-line attack of innate immune components such as phagocytes and the complement system. Indeed, these innate immune components play a major role both in the control and establishment of *L. donovani* infections [28]. Complement components including lectins are the primary molecules of the innate immune system to encounter inoculated metacyclic promastigotes. The early activation of the complement system during pathogen invasion occurs predominantly by the lectin pathway, as it is independent of a specific antibody response. Moreover, it prompts the activation of the alternative pathway [29]. The lectin pathway protein MBL induces opsonophagocytosis by depositing C3b on the surface of *Leishmania* which is crucial for parasite survival and multiplication [13,14]. We assume that, as ficolins are functionally similar to MBL, they equally influence the outcome of VL. No study so far, however,

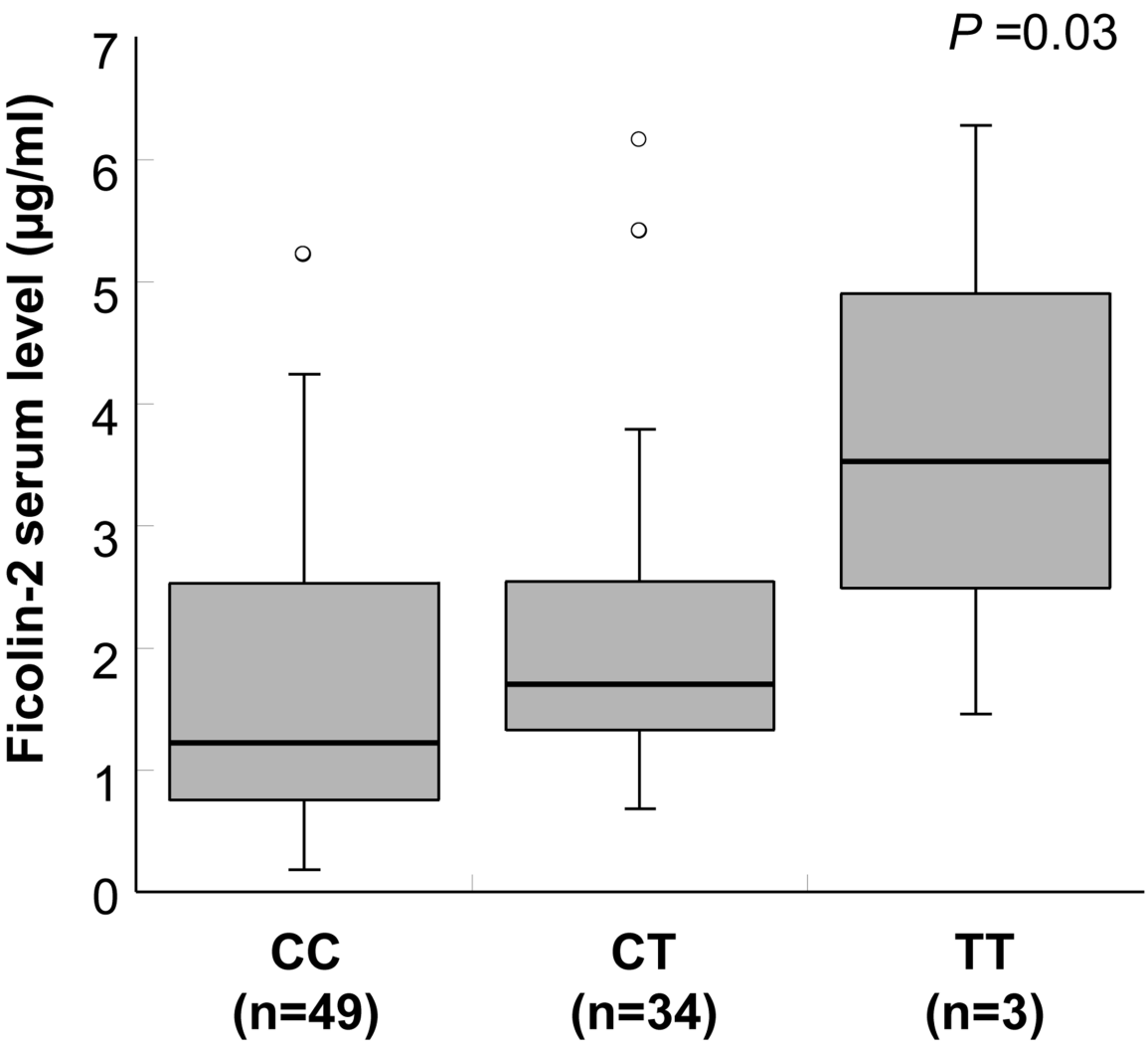

Fig 3. Distribution of ficolin-2 serum levels with +6359C>T variant in controls. Box-plots illustrate medians with 25 and 75 percentiles with whiskers to 10 and 90 percentiles. Ficolin-2 serum levels were measured and separated based on different genotypes of *FCN2* variant +6359C>T. *P* = 0.03 illustrated in the figure is calculated by Kruskal-Wallis rank sum test.

doi:10.1371/journal.pone.0125940.g003

has focused on the role of ficolin-2 in VL. We studied the contribution of ficolin-2 serum levels and of *FCN2* functional variants in VL.

The structural variant +6359C>T (p.T236M) in the fibrinogen-like domain of the *FCN2* gene confers relative susceptibility to VL. The finding remains consistent in recessive and allelic genetic models. The computational prediction revealed that the T236M substitution has a major impact on the physiochemical property of ficolin-2 [30]. In addition, the *+6359T* allele was found associated with higher ficolin-2 serum levels [31] and the observation was reconfirmed in a cohort of neonates [32]. We also observed a similar effect of the *+6359T* allele in controls, but not in cases. Our results inferred that ficolin-2 serum levels were modulated significantly by the infection in VL cases rather than by *FCN2* variants. Moreover, the ficolin-2 protein with T236M substitution had a markedly decreased binding capacity to acetylated agarose beads. Therefore, this structural variant is believed to alter the binding properties of the protein to recognize invading pathogens [24,33,34]. These reports indicate that individuals with higher ficolin-2 serum levels and altered binding capacities might favor *L. donovani*

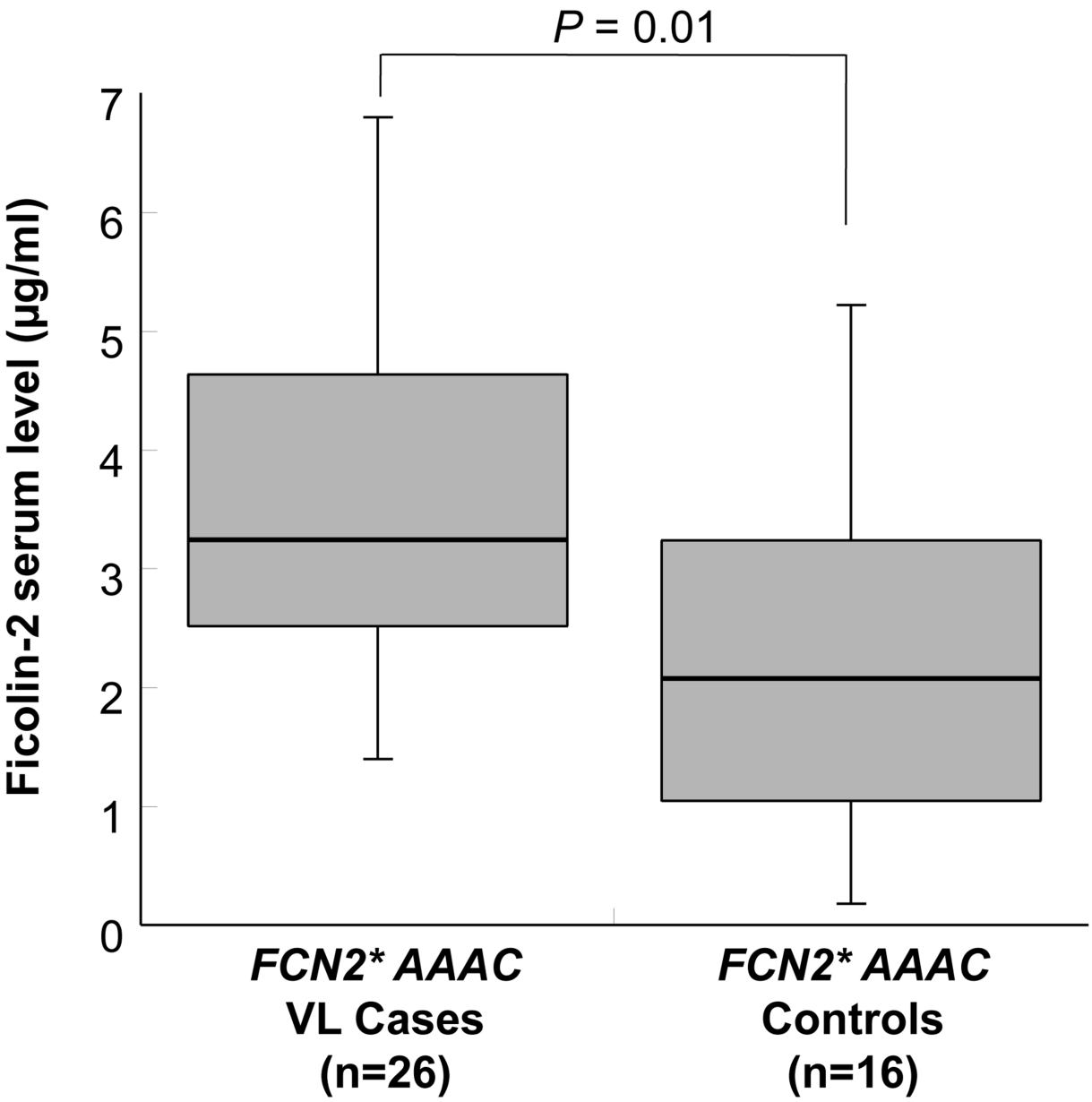

Fig 4. Distribution of ficolin-2 serum levels with *FCN2*AAAC* in VL cases and control. Box-plots illustrate medians with 25 and 75 percentiles with whiskers to 10 and 90 percentiles. *P* = 0.01 illustrated in the figure is calculated by Wilcoxon-Mann-Whitney rank sum test.

doi:10.1371/journal.pone.0125940.g004

invasion into macrophages and the development of VL. Previous studies have also reported that +6359C>T in the *FCN2* gene is a risk factor for staphylococcal peritonitis in continuous ambulatory peritoneal dialysis cases [35] and for bloodstream infections in kidney transplant recipients [36].

In *FCN2* gene-association studies, haplotype analyses should be taken into account as they may influence disease susceptibility [37]. Our *FCN2* haplotypes revealed that the *FCN2*AAAC* haplotype frequency was higher among controls than in VL cases, indicating that individuals with this haplotype had a diminished probability to develop VL. The *FCN2*AAAC* haplotype harbors the *+6359C* major allele, which accounts for reduced ficolin-2 levels [31,32]. In light of

these observations, it is evident that the *FCN2* genetic factors that contribute to low ficolin-2 level decrease the risk of VL. *FCN2* promoter haplotypes did not show any differences among groups, suggesting the relative contribution of the +6359C>T genotype in Indian VL.

Ficolin-2 serum levels were elevated in VL cases compared to controls, indicating that ficolin-2 is a susceptibility factor. The result is in accordance with a study published previously [15]. Corresponding results were also observed in infections with *Mycobacterium* spp., where higher MBL serum levels increased the risk of infection [38–40]. The proposed mechanism may be that intracellular parasites abuse C3 opsonization and enhance opsonaophagocytosis by monocytes/macrophages to avoid complement attacks. Any increase in the MBL and ficolin levels in turn may enhance complement activation and, thus, the probability of parasitization by depositing C3b on parasite surfaces [41]. Our observation supports this notion as cases with VL had higher ficolin-2 levels than uninfected controls. Nevertheless, discordant results were reported for ficolin-2 in tuberculosis and Chagas disease, where cases presented lower ficolin-2 plasma levels than did controls [22,42]. No clear mechanism is proposed to address the conflictive observations of functionally similar proteins in intracellular habitant infections. In addition, the recognition and interaction of mannose binding lectin (MBL) with *Leishmania* parasites are well established [13,14] and ficolins were shown to be functional analogous to MBL [16]. However, a limitation of our study is that there is a lack of data showing the interaction of ficolin-2 with *L. donovani*. Nevertheless, our earlier study demonstrated the genetic association of *FCN2* polymorphism with cutaneous leishmaniasis in Syrian population [17].

In conclusion, our results show that the *FCN2* +6359C>T variant is associated with increased susceptibility to VL and that the *FCN2*AAAC* haplotype is associated with relative protection. Higher serum ficolin-2 levels were observed in cases with VL than among controls.

Acknowledgments

We acknowledge the help of Dr. B.N. Jha (District Hospital, Muzaffarpur), Dr. Gyan Prakash (CMO, Muzaffarpur) and the subjects, who have voluntarily participated in this study.

Author Contributions

Conceived and designed the experiments: KT TPV. Performed the experiments: AM JSA PS RDJ. Analyzed the data: JSA HVT TPV. Contributed reagents/materials/analysis tools: AM KT TPV. Wrote the paper: JSA CGM TPV KT.

References

1. Ready PD (2014) Epidemiology of visceral leishmaniasis. Clin Epidemiol 6: 147–154. doi: 10.2147/CLEP.S44267 [doi];clep-6-147 [pii]. PMID: 24833919
2. Mathers CD, Ezzati M, Lopez AD (2007) Measuring the burden of neglected tropical diseases: the global burden of disease framework. PLoS neglected tropical diseases 1: e114. doi: 10.1371/journal.pntd.0000114 PMID: 18060077
3. Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, et al. (2007) Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nature reviews Microbiology 5: 873–882. doi: 10.1038/nrmicro1748 PMID: 17938629
4. Desjeux P (1996) Leishmaniasis. Public health aspects and control. Clinics in dermatology 14: 417–423. PMID: 8889319
5. Hotez PJ, Remme JH, Buss P, Alleyne G, Morel C, Breman JG (2004) Combating tropical infectious diseases: report of the Disease Control Priorities in Developing Countries Project. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 38: 871–878. doi: 10.1086/382077
6. Singh SP, Reddy DC, Rai M, Sundar S (2006) Serious underreporting of visceral leishmaniasis through passive case reporting in Bihar, India. Tropical medicine & international health: TM & IH 11: 899–905. doi: 10.1111/j.1365-3156.2006.01647.x

7. Muniaraj M (2014) The lost hope of elimination of Kala-azar (visceral leishmaniasis) by 2010 and cyclic occurrence of its outbreak in India, blame falls on vector control practices or co-infection with human immunodeficiency virus or therapeutic modalities? Tropical parasitology 4: 10–19. doi: 10.4103/2229-5070.129143 PMID: 24754021
8. Sacks D, Kamhawi S (2001) Molecular aspects of parasite-vector and vector-host interactions in leishmaniasis. Annual review of microbiology 55: 453–483. doi: 10.1146/annurev.micro.55.1.453 PMID: 11544364
9. Moradin N, Descoteaux A (2012) Leishmania promastigotes: building a safe niche within macrophages. Frontiers in cellular and infection microbiology 2: 121. doi: 10.3389/fcimb.2012.00121 PMID: 23050244
10. Descoteaux A, Turco SJ (1999) Glycoconjugates in Leishmania infectivity. Biochimica et biophysica acta 1455: 341–352. PMID: 10571023
11. Flandin JF, Chano F, Descoteaux A (2006) RNA interference reveals a role for TLR2 and TLR3 in the recognition of Leishmania donovani promastigotes by interferon-gamma-primed macrophages. European journal of immunology 36: 411–420. doi: 10.1002/eji.200535079 PMID: 16369915
12. Descoteaux A, Turco SJ (2002) Functional aspects of the Leishmania donovani lipophosphoglycan during macrophage infection. Microbes and infection / Institut Pasteur 4: 975–981. PMID: 12106791
13. Ambrosio AR, De Messias-Reason IJ (2005) Leishmania (Viannia) braziliensis: interaction of mannose-binding lectin with surface glycoconjugates and complement activation. An antibody-independent defence mechanism. Parasite immunology 27: 333–340. doi: 10.1111/j.1365-3024.2005.00782.x PMID: 16149991
14. Green PJ, Feizi T, Stoll MS, Thiel S, Prescott A, McConville MJ (1994) Recognition of the major cell surface glycoconjugates of Leishmania parasites by the human serum mannan-binding protein. Molecular and biochemical parasitology 66: 319–328. PMID: 7808481
15. Santos IK, Costa CH, Krieger H, Feitosa MF, Zurakowski D, Fardin B et al. (2001) Mannan-binding lectin enhances susceptibility to visceral leishmaniasis. Infection and immunity 69: 5212–5215. doi: 10.1128/IAI.69.8.5212-5215.2001 PMID: 11447210
16. Ren Y, Ding Q, Zhang X (2014) Ficolins and infectious diseases. Virologica Sinica 29: 25–32. doi: 10.1007/s12250-014-3421-2 PMID: 24452543
17. Assaf A, Hoang TV, Faik I, Aebischer T, Kremsner PG, Kun JF et al. (2012) Genetic evidence of functional ficolin-2 haplotype as susceptibility factor in cutaneous leishmaniasis. PloS one 7: e34113. doi: 10.1371/journal.pone.0034113 PMID: 22457818
18. Matsushita M (2010) Ficolins: complement-activating lectins involved in innate immunity. Journal of innate immunity 2: 24–32. doi: 10.1159/000228160 PMID: 20375620
19. Matsushita M (2013) Ficolins in complement activation. Molecular immunology 55: 22–26. doi: 10.1016/j.molimm.2012.08.017 PMID: 22959617
20. Hoang TV, Toan NL, Song lH, Ouf EA, Bock CT, Kremsner PG et al. (2011) Ficolin-2 levels and FCN2 haplotypes influence hepatitis B infection outcome in Vietnamese patients. PLoS One 6: e28113. doi: 10.1371/journal.pone.0028113 [doi];PONE-D-11-14849 [pii]. PMID: 22140517
21. Ouf EA, Ojurongbe O, Akindele AA, Sina-Agbaje OR, Van TH, Adeyeba AO et al. (2012) Ficolin-2 levels and FCN2 genetic polymorphisms as a susceptibility factor in schistosomiasis. J Infect Dis 206: 562–570. jis396 [pii];doi: 10.1093/infdis/jis396 [doi]. PMID: 22693230
22. Luz PR, Boldt AB, Grisbach C, Kun JF, Velavan TP, Messias-Reason IJ (2013) Association of L-ficolin levels and FCN2 genotypes with chronic Chagas disease. PloS one 8: e60237. doi: 10.1371/journal.pone.0060237 PMID: 23593180
23. Endo Y, Matsushita M, Fujita T (2011) The role of ficolins in the lectin pathway of innate immunity. The international journal of biochemistry & cell biology 43: 705–712. doi: 10.1016/j.biocel.2011.02.003
24. Hummelshoj T, Munthe-Fog L, Madsen HO, Fujita T, Matsushita M, Garred P (2005) Polymorphisms in the FCN2 gene determine serum variation and function of Ficolin-2. Human molecular genetics 14: 1651–1658. doi: 10.1093/hmg/ddi173 PMID: 15879437
25. Munthe-Fog L, Hummelshoj T, Hansen BE, Koch C, Madsen HO, Skjodt K et al. (2007) The impact of FCN2 polymorphisms and haplotypes on the Ficolin-2 serum levels. Scandinavian journal of immunology 65: 383–392. doi: 10.1111/j.1365-3083.2007.01915.x PMID: 17386030
26. Krarup A, Thiel S, Hansen A, Fujita T, Jensenius JC (2004) L-ficolin is a pattern recognition molecule specific for acetyl groups. The Journal of biological chemistry 279: 47513–47519. doi: 10.1074/jbc.M407161200 PMID: 15331601
27. Thangaraj K, Joshi MB, Reddy AG, Gupta NJ, Chakravarty B, Singh L (2002) CAG repeat expansion in the androgen receptor gene is not associated with male infertility in Indian populations. J Androl 23: 815–818. PMID: 12399527

28. Laurenti MD, Corbett CE, Sotto MN, Sinhorini IL, Goto H (1996) The role of complement in the acute inflammatory process in the skin and in host-parasite interaction in hamsters inoculated with Leishmania (Leishmania) chagasi. International journal of experimental pathology 77: 15–24. PMID: 8664142

29. Cestari I, Evans-Osses I, Schlapbach LJ, de Messias-Reason I, Ramirez MI (2013) Mechanisms of complement lectin pathway activation and resistance by trypanosomatid parasites. Molecular immunology 53: 328–334. doi: 10.1016/j.molimm.2012.08.015 PMID: 23063472

30. Hummelshoj T, Munthe-Fog L, Madsen HO, Garred P (2008) Functional SNPs in the human ficolin (FCN) genes reveal distinct geographical patterns. Molecular immunology 45: 2508–2520. doi: 10.1016/j.molimm.2008.01.003 PMID: 18289682

31. Cedzynski M, Nuytinck L, Atkinson AP, St Swierzko A, Zeman K, Szemraj J et al. (2007) Extremes of L-ficolin concentration in children with recurrent infections are associated with single nucleotide polymorphisms in the FCN2 gene. Clinical and experimental immunology 150: 99–104. doi: 10.1111/j.1365-2249.2007.03471.x PMID: 17680820

32. Kilpatrick DC, St Swierzko A, Matsushita M, Domzalska-Popadiuk I, Borkowska-Klos M, Szczapa J et al. (2013) The relationship between FCN2 genotypes and serum ficolin-2 (L-ficolin) protein concentrations from a large cohort of neonates. Human immunology 74: 867–871. doi: 10.1016/j.humimm.2013.04.011 PMID: 23619474

33. Herpers BL, Immink MM, de Jong BA, van Velzen-Blad H, de Jongh BM, van Hannen EJ (2006) Coding and non-coding polymorphisms in the lectin pathway activator L-ficolin gene in 188 Dutch blood bank donors. Molecular immunology 43: 851–855. doi: 10.1016/j.molimm.2005.06.035 PMID: 16076493

34. Ma YJ, Doni A, Hummelshoj T, Honore C, Bastone A, Mantovani A et al. (2009) Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. The Journal of biological chemistry 284: 28263–28275. doi: 10.1074/jbc.M109.009225 PMID: 19632990

35. Meijvis SC, Herpers BL, Endeman H, de Jong B, van Hannen E, van Velzen-Blad H et al. (2011) Mannose-binding lectin (MBL2) and ficolin-2 (FCN2) polymorphisms in patients on peritoneal dialysis with staphylococcal peritonitis. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association—European Renal Association 26: 1042–1045. doi: 10.1093/ndt/gfq474

36. Wan QQ, Ye QF, Zhou JD (2013) Mannose-binding lectin 2 and ficolin-2 gene polymorphisms influence the susceptibility to bloodstream infections in kidney transplant recipients. Transplantation proceedings 45: 3289–3292. doi: 10.1016/j.transproceed.2013.05.008 PMID: 24182802

37. de Messias-Reason I, Kremsner PG, Kun JF (2009) Functional haplotypes that produce normal ficolin-2 levels protect against clinical leprosy. The Journal of infectious diseases 199: 801–804. PMID: 19434912

38. De Messias-Reason IJ, Boldt AB, Moraes Braga AC, Von Rosen Seeling Stahlke E, Dornelles L, Pereira-Ferrari L et al. (2007) The association between mannan-binding lectin gene polymorphism and clinical leprosy: new insight into an old paradigm. The Journal of infectious diseases 196: 1379–1385. doi: 10.1086/521627 PMID: 17922403

39. Hoal-Van Helden EG, Epstein J, Victor TC, Hon D, Lewis LA, Beyers N et al. (1999) Mannose-binding protein B allele confers protection against tuberculous meningitis. Pediatric research 45: 459–464. doi: 10.1203/00006450-199904010-00002 PMID: 10203135

40. Soborg C, Madsen HO, Andersen AB, Lillebaek T, Kok-Jensen A, Garred P (2003) Mannose-binding lectin polymorphisms in clinical tuberculosis. The Journal of infectious diseases 188: 777–782. doi: 10.1086/377183 PMID: 12934195

41. Dommett RM, Klein N, Turner MW (2006) Mannose-binding lectin in innate immunity: past, present and future. Tissue Antigens 68: 193–209. TAN649 [pii];doi: 10.1111/j.1399-0039.2006.00649.x [doi]. PMID: 16948640

42. Luo F, Sun X, Wang Y, Wang Q, Wu Y, Pan Q et al. (2013) Ficolin-2 defends against virulent Mycobacteria tuberculosis infection in vivo, and its insufficiency is associated with infection in humans. PloS one 8: e73859. doi: 10.1371/journal.pone.0073859 PMID: 24040095

VOLUME 20 NO 10 PP 1311–1319 OCTOBER 2015

Low MBL-associated serine protease 2 (MASP-2) levels correlate with urogenital schistosomiasis in Nigerian children

Olusola Ojurongbe[1,2,†], Justin S. Antony[1,†], Hoang Van Tong[1], Christian G. Meyer[1], Akeem A. Akindele[2], Olawumi R. Sina-Agbaje[3], Peter G. Kremsner[1] and Thirumalaisamy P. Velavan[1,4]

1 *Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany*
2 *Ladoke Akintola University of Technology, Ogbomoso, Nigeria*
3 *Ogun State Hospital, Ijaye Abeokuta, Nigeria*
4 *Fondation Congolaise pour la Recherche Medicale, Brazzaville, Republic of Congo*

Abstract

OBJECTIVES The human mannose-binding lectin (MBL) and ficolins (FCN) are involved in pathogen recognition in the first line of defence. They support activation of the complement lectin cascade in the presence of MBL-associated serine protease 2 (MASP-2), a protein that cleaves the C4 and C2 complement components. Recent studies found that distinct *MBL2* and *FCN2* promoter variants and their corresponding serum levels are associated with relative protection from urogenital schistosomiasis.

METHODS We investigated the contribution of MASP-2 levels and *MASP2* polymorphisms in a Nigerian study group, of 163 individuals infected with *Schistosoma haematobium* and 183 healthy subjects.

RESULTS MASP-2 serum levels varied between younger children (≤12 years) and older children (>12 years) and adults ($P = 0.0001$). Younger children with a patent infection had significantly lower MASP-2 serum levels than uninfected children ($P = 0.0074$). Older children and adults (>12 years) with a current infection had higher serum MASP-2 levels than controls ($P = 0.032$). MBL serum levels correlated positively with MASP-2 serum levels ($P = 0.01$). *MASP2* secretor haplotypes were associated with MASP-2 serum levels in healthy subjects. The heterozygous *MASP2* p.P126L variant was associated with reduced serum MASP-2 levels ($P = 0.01$).

CONCLUSIONS The findings indicate that higher MASP-2 serum levels are associated with relative protection from urogenital schistosomiasis in Nigerian children.

keywords MASP-2, MBL, complement, genotypes, haplotypes, Schistosomiasis

Introduction

Schistosomiasis is caused by several trematode species of the genus *Schistosoma* and is one of the major neglected tropical diseases targeted for elimination by WHO [1]. Schistosomiasis is a considerable public health problem in sub-Saharan Africa, and Nigeria shares 14% of the global schistosomiasis burden [2]. The most widely distributed species in many parts of Nigeria is *S. haematobium*. The parasite causes immense morbidity as assessed by disability-adjusted life-years. An enormous prevalence of schistosomiasis occurs among school children who are frequently in contact with water infested by the infectious form of schistosoma, the cercariae [1, 3]. The disease may lead to severe pathological conditions such as haematuria, hydronephrosis and bladder cancer [1, 4]. The limited availability of praziquantel [5], the drug of choice in schistosomiasis treatment, rapid and frequent reinfections and failure of the immune system to clear infections adds to the schistosomiasis burden [6].

Schistosomes are multicellular helminth parasites bearing an outer syncytial cytoplasmic layer, the tegument [7]. Schistosomal teguments consist of fucosylated carbohydrate epitopes [8] and glycoproteins [9] which are expressed at all developmental stages. These glycoconjugates serve as pathogen-associated molecular patterns (PAMPs) in immune recognition and subsequent complement-mediated lysis [10, 11]. Previous *in vitro* studies have shown that mannose-binding lectin (MBL) binds to the sugar moieties of *S. mansoni* cercariae and adult worms together with the MBL-associated serine protease 1 (MASP-1) and MASP-2 and subsequently activates the complement cascade [12].

[†]Contributed equally to the work.

MBL and ficolins (ficolin-1, ficolin-2 and ficolin-3) are pattern recognition molecules (PRMs). Variants of the genes encoding these molecules are associated with several infectious diseases [13–17]. The PRMs bind to the surface glycoconjugates and form a complex with MASPs (MASP-1, MASP-2 and MASP-3) to proteolytically cleave the circulating complement components C4 and C2 to form C3 convertase (C4b2a) [18]. The C3 convertase subsequently activates the lectin complement cascade, which eventually results in the membrane attack complex and lysis of the pathogen [19]. Currently, the precise role of MASP-3 in the lectin complement pathway is not well established [20]. However, MASP-1 and MASP2 have been proven to be functional in the cleavage of C4 and C2. MASP-2 is therefore a vital protein in the lectin complement cascade [21].

The gene-encoding MASP-2 (*MASP2*) is located on chromosome 1p31.23-31 (OMIM: 605102) and comprises 12 exons. MASP-2 is composed of six domains including C1r/C1s, Uegf and bone morphogenetic protein 1 domain (CUB1), epidermal growth factor, CUB2, contiguous complement control proteins 1 and 2 and a serine protease domain [22]. It was shown that single nucleotide polymorphisms (SNPs) of *MASP2* are associated with circulating MASP-2 serum levels [23, 24]. The non-synonymous variant of *MASP2* (rs72550870; p.D120G) located in the CUB1 domain prevents formation of the ficolin/MASP-2 complex, causing the complement cascade to fail. In addition, the non-synonymous *MASP2* substitutions p.P126L (rs56392418) and p.R99Q (rs61735600) in the CUB1 domain were reported to be associated with altered MASP-2 levels [25]. Ten *MASP2* haplotypes have so far been identified and shown to modulate circulating MASP-2 levels. The nomenclature of the haplotypes accounts for the variable capability of MASP-2 secretion, classifying them as high secretor (*MASP-2*1A, *1B1-h*, **1B2-h*), intermediate secretor (*MASP-2*2B1-i, *2B2A-i*) and low MASP-2 secretor haplotypes (*MASP-2*1C1-l, *1C2-l,*2A1,*2A2-l* and **2B2B*-l) [23].

The functional role of *MASP2* variants and/or MASP-2 levels has been addressed in a few disease association studies only, namely in Chagas disease, leprosy, placental malaria, rheumatic fever and hepatitis C virus infection [26–30]. Recent studies have indicated that distinct *MBL2* and *FCN2* promoter variants and their corresponding serum levels are associated with relative protection from urogenital schistosomiasis [31, 32]. MASP-2 is a protein that occurs downstream lectin complement pathway and the generation of C3 convertase, and it is crucial for the complement lectin cascade. Therefore, we investigated circulating MASP-2 serum levels and previously reported *MASP2* genetic variants in the promoter region (1), exon 3 (3), intron 9 (1), exon 10 (2) and exon 11 (2) for associations with clinically patent urogenital schistosomiasis [26, 27, 33].

Materials and methods

Study design and sample collection

We recruited 346 individuals, irrespective of their infection status from two communities of south-west Nigeria where *S. haematobium* infection is endemic (Ilewo Orile, Abeokuta North and Ore, Osogbo). All participants were of Yoruba ethnicity. About ten mL of urine was collected from all participants for microscopic examination of *S. haematobium* eggs. In brief, urine samples were centrifuged and the sediment was examined microscopically for the presence of *S. haematobium* eggs. For negative individuals, urine samples were collected on three successive days to ensure negativity. A Combur-Test reagent strip (Roche Diagnostics, Mannheim, Germany) was used to estimate the haematuria and proteinuria. About 5 mL of full venous blood was collected from all study participants for DNA extraction and serological assays. Persons positive for urogenital schistosomiasis were treated with a single dose of 40 mg/kg praziquantel. Stool samples were collected from all participants and processed using the Kato-Katz method to exclude *S. mansoni* infection. Detailed information on the study group and the study design is provided elsewhere [31, 32].

After parasitological and serological testing (anti-Schistosoma total IgG antibodies as marker of exposure), the study group was divided into three groups. The case group, defined as positive for *S. haematobium* eggs in urine (Schistosoma egg positive (SEP); $n = 163$), consisted of 99 (61%) children, 90 (55%) of them males and 73 (45%) females. The mean age was 17 (±12.4), and 90% of them had haematuria. The first control group was defined as being negative for *S. haematobium* eggs in urine, but positive for *Schistosoma* total IgG antibodies (Schistosoma ELISA positive (SELP); $n = 118$). Of these, 58 (50%) were males and 60 (50%) were females; 23 (19%) were children under 12. The mean age in this group was 34.3 (±19.1). The second control group was defined as being negative for *S. haematobium* eggs in urine and negative for anti-Schistosoma total IgG antibodies (Schistosoma ELISA negative, (SELN); $n = 65$). A total of 31 (48%) individuals were children, 40 (62%) were males and 25 (38%) were females; the mean age was 20.2 (±17.2).

Serological assays

Serological assays applying an in-house ELISA system [34] were carried out to detect total anti-*Schistosoma* IgG

antibodies. The technique has been described previously in detail [32]. MASP-2 serum levels were measured in all study subjects using the commercially available human MASP-2 ELISA kit (Hycult Biotech, Uden, the Netherlands) following the manufacturer's instructions. The lower detection limit of the assay was 1.6 ng/ml.

MASP2 genotyping

Genomic DNA was extracted from peripheral blood cells using the QIAamp DNA mini blood kit (Qiagen, Hilden, Germany). The fragment containing nine studied *MASP2* variants was PCR-amplified from three genomic regions. The promoter and exon 3 were amplified by primer pairs PromF: 5′-TGAAGCCGGGGCAACAGAACA-3′ and Exon3R: 5′-ACAGAGTTACCCCCACAGCCAG-3′, while intron 9 and exon 10 were amplified by primers Int9F: 5′-TGTTAGCCAGGATGGTCTCC-3′ and Exon10R: 5′-GGGGGCTCAAGTTCCAAGTA-3′ and the exon 12 was amplified by primers Exon12F: 5′-TCAGGTGTTT-GAACTGTGATGTT-3′ and Exon12R: 5′-GGCAGACAAATAGGCGTGAT-3′. In brief, 10 ng of genomic DNA was amplified in a 20 μl volume of reaction mixture containing 1x PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM of $MgCl_2$), 0.125 mM of dNTPs, 0.25 mM of each primer and 1U Taq DNA polymerase (Qiagen, Hilden, Germany) on a PTC-200 Thermal cycler (MJ Research, Waltham, USA). Thermal cycling parameters for amplification of all fragments were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 45 sec at 94 °C denaturation, 45 s annealing at 68 °C for the promoter and exon 3, 66 °C for the intron 9 and exon 10 and 61.5 °C for the exon 12. Extension was 1 min 30 s at 72 °C, followed by a final extension step of 2 min at 72 °C. Subsequently, PCR products were purified using Exo-SAP-IT (USB Affymetrix, Santa Clara, USA), and 1 μl of the purified product was directly used as template for sequencing (BigDye terminator v. 1.1 cycle sequencing kit; Applied Biosystems, Foster City, CA, USA) on an ABI 3130XL DNA sequencer. For amplicons generated from the promoter and exon 3 regions, internal primers were used for sequencing. The internal reverse primer 5′-CTACGCTGGTCTCACCTTTGAT-3′ was used for the -154C>A (rs7548659) variant and an additional internal exon 1 forward primer 5′-CGCCTCTACTTCACC-CACTTCG-3′ was used for sequencing variants in the codons p.R99Q, p.D120G and p.P126L. Polymorphisms were identified by assembling the sequences with respective reference sequences of the *MASP2* gene (NG_007289.1) using Codoncode Aligner 4.0 software (http://www.codoncode.com/) and were reconfirmed visually from their electropherograms.

Statistical analysis

Data were analysed using the STATA software (Intercooled STATA; STATA Corp., College Station, TX, USA), and the level of significance was set to a *P*-value of <0.05. Two-sided Fisher exact tests were computed to determine the distribution of the genotypes and alleles between cases and control groups. Bonferroni corrections applied. Multivariate analysis was performed to adjust the confounding effects of age and gender in estimations of the contribution of *MASP2* variant and MASP-2 serum levels to schistosomiasis susceptibility. Kruskal–Wallis or Wilcoxon–Mann–Whitney rank sum tests were applied to analyse associations of serum MASP-2 levels with *MASP2* variants, haplotypes and phenotypes using the KaleidaGraph software (www.synergy.com). Genotype and haplotype frequencies were calculated by simple gene counting and by expectation-maximum (EM) algorithm. Possible deviations from Hardy–Weinberg equilibrium were tested using the random permutation procedure as implemented in the Arlequin v.3.5.1.2 software (http://lgb.unige.ch/arlequin). Linkage disequilibrium (LD) analyses were performed using Haploview v.3.2 (http://broadinstitute.org/haploview). The correlation between serum MBL and ficolin-2 levels with serum MASP-2 levels was analysed by nonparametric Spearman's rank coefficient using the SPSS statistical package. The MBL and ficolin-2 data have been described previously [31, 32].

Ethics statement

Informed consent was obtained from all participants; for children, consent was obtained from their parents and/or guardians. Ethical approval was sought for and obtained from the Ethical Committee of the Ministry of Health, Abeokuta Ogun State, Nigeria.

Results

MASP2 gene polymorphisms and urogenital schistosomiasis

Both genotype and allele frequencies for all analysed functional *MASP2* variants in each subgroup were in Hardy–Weinberg equilibrium, except for the variant rs2273346 (p.V377A) in SEP cases, which was excluded from further analyses. LD patterns of the nine *MASP2* variants studied in the case (SEP) group, the previously exposed (SELP) group, the non-exposed (SELN group) and the combined controls (SELP and SELN) are shown in Figure 1. The distribution of *MASP2* alleles and genotypes in the entire study group is given in Table 1. No

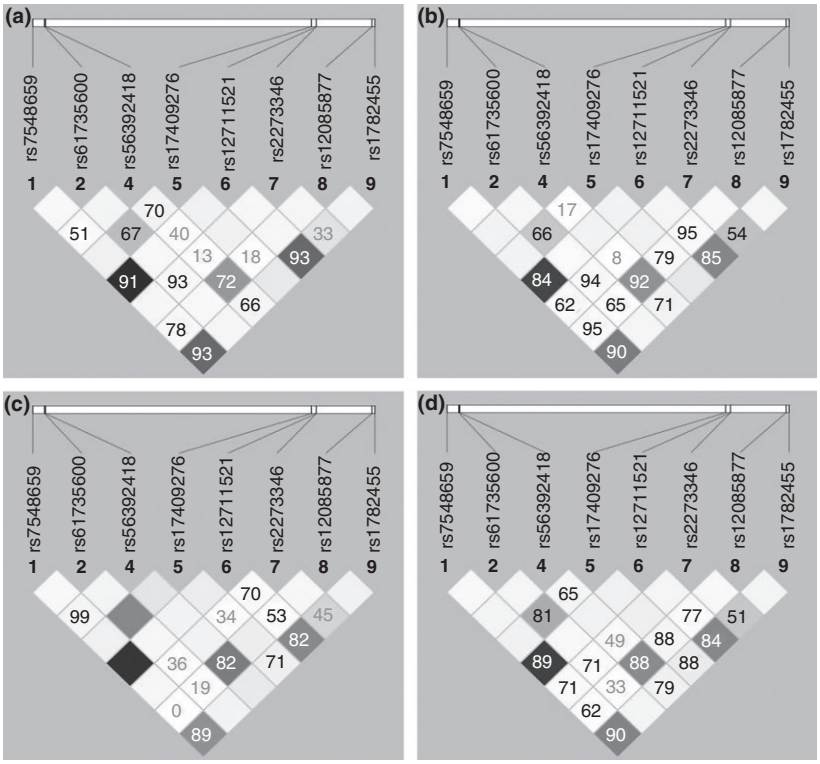

Figure 1 (a) Linkage disequilibrium patterns of studied SNPs in cases who tested positive for *Schistosoma* eggs, the SEP group; (b) individuals who tested positive for *Schistosoma* antigens by ELISA, the SELP group (control group 1); (c) individuals who tested negative for *Schistosoma* antigens by ELISA and negative for *Schistosoma* eggs, the SELN group (control group 2); and (d) the SELP and SELN groups combined. Empty squares indicate a high degree of linkage disequilibrium (D' = 1). Numbers indicate the D′ value expressed as a percentile. The dense shading indicates the r^2 value.

significant contribution of alleles or genotypes was observed. Although a marginal contribution of the rs56392418 (p.P126L) variant was seen (SEP cases vs. SELP+SELN controls OR 6.9; 95% CI 0.8–321.5; $P = 0.04$), the observation was not significant after testing for multiple comparisons. The exon 3 variant rs72550870 (p.D120G*)* was monomorphic in the population under study.

MASP2 haplotypes and urogenital schistosomiasis

A total of twelve haplotypes were observed at frequencies >2% in our study population (data not shown). The frequencies of the secretor haplotypes observed did not differ between subgroups (SEP cases vs. SELP, SELN controls; SEP cases vs. SELP+SELN controls).

MASP-2 serum levels and urogenital schistosomiasis

MASP-2 serum levels varied from 1.8 to 1073 ng/ml with a median of 106.9 ng/ml. The serum levels were equally distributed among our study subgroups ($P > 0.05$). The multivariate analysis, however, revealed a significant contribution of age to MASP-2 serum levels ($P = 0.0001$). We therefore grouped our study participants according to age and to the rationale that younger children might be rather at risk, as they have frequent contacts with water infested by the infectious form of schistosoma the cercariae. Younger children below the puberty age are more susceptible to schistosomiasis than older children and adults [35]. Based on an average puberty age of 12 years in the population studied [36], the subjects were separated into the groups of ≤12 years and >12 years of age. We observed that MASP-2 serum levels were significantly lower in children ≤12 years with a patent infection (SEP) than in those with previous infections (SELP) ($P = 0.0074$; Figure 2a). MASP-2 serum levels were significantly higher in infected (SEP) older children and adults (>12 years) than in those who were exposed previously (SELP) ($P = 0.032$; Figure 2b). We also correlated the MBL and ficolin-2 serum levels described previously for the same study group [31, 32] with the circulating MASP-2 serum levels assessed here. Only MBL serum levels were positively correlated with MASP-2 serum levels ($r = 0.39$, $P = 0.01$; Figure 3a).

MASP2 gene polymorphism and MASP-2 serum levels

MASP-2 serum levels did not differ with regard to high, intermediate or low secretor haplotypes, neither in active

Table 1 Distribution of *MASP2* genotypes and allele(s) among cases and controls

							SEP vs. SELP		SEP vs. (SELP+SELN)	
	SNP position	Genotype	SEP *n* = 163 (%)	SELP *n* = 118 (%)	SELN *n* = 65 (%)	SELP+SELN *n* = 183 (%)	OR (95% CI)	*P*-value	(95% CI)	*P*-value
Prom (C>A)	rs7548659	*CC*	126 (77.3)	92 (78)	52 (80)	144 (78.7)				
		CA	36 (22.1)	23 (19.5)	13 (20)	36 (19.7)		NS		NS
		AA	1 (0.6)	3 (2.5)	0	3 (1.6)				
		C	288 (88.3)	207 (87.7)	117 (90)	324 (88.5)				
		A	38 (11.7)	29 (12.3)	13 (10)	42 (11.5)				
Exon3(G>A)	rs61735600 (R99Q)	*GG*	133 (81.6)	101 (85.6)	51 (78.5)	152 (83.1)				
		GA	30 (18.4)	17 (14.4)	14 (21.5)	31 (16.9)		NS		NS
		AA	0	0	0	0				
		G	296 (90.8)	219 (92.8)	116 (89.2)	335 (91.5)				
		A	30 (9.2)	17 (7.2)	14 (10.8)	31 (8.5)				
Exon3(A>G)	rs72550870 (D120G)	*AA*	163 (100)	118 (100)	65 (100)	183 (100)				
		AG	0	0	0	0		NS		NS
		GG	0	0	0	0				
		A	326 (100)	236 (100)	130 (100)	366 (100)				
		G	0	0	0	0				
Exon 3(C>T)	rs56392418 (P126L)	*CC*	106 (65)	76 (64.4)	48 (73.8)	124 (67.8)		NS		NS
		CT	51 (31.3)	42 (35.6)	16 (24.6)	58 (31.7)		NS		NS
		TT	6 (3.7)	0 1 (1.5)	1 (0.5)	NS		NS		NS
		C	263 (80.7)	194 (82.2)	112 (86.2)	306 (83.6)				
		T	63 (19.3)	42 (17.8)	18 (13.8)	60 (16.4)				
Intron9(C>T)	rs1961795	*CC*	106 (65)	78 (66.1)	38 (58.5)	116 (63.4)				
		CT	51 (31.3)	38 (32.2)	23 (35.4)	61 (33.3)		NS		NS
		TT	6 (3.7)	2 (1.7)	4 (6.2)	6 (3.3)				
		C	263 (80.7)	194 (82.2)	99 (76.2)	293 (80.1)				
		T	63 (19.3)	42 (17.8)	31 (23.8)	73 (19.9)				
Exon 10(G>T)	rs12711521 (D371Y)	*GG*	126 (77.3)	94 (79.7)	50 (76.9)	144 (78.7)				
		GT	36 (22.1)	19 (16.1)	14 (21.5)	33 (18)		NS		NS
		TT	1 (0.6)	5 (4.2)	1 (1.5)	6 (3.3)				
		G	288 (88.3)	207 (87.7)	114 (87.7)	321 (87.7)				
		T	38 (11.7)	29 (12.3)	16 (12.3)	45 (13.1)				
Exon 10(T>C)	rs2273346 (V377A)	*TT*	127 (77.9)	87 (73.7)	49 (75.4)	136 (74.3)				
		TC	30 (18.4)	30 (25.4)	16 (24.6)	46 (25.1)		NA		NA
		CC	6 (3.7)	1 (0.8)	0	1 (0.5)				
		T	284 (87.1)	204 (86.4)	114 (87.7)	318 (86.9)				
		C	42 (12.9)	32 (13.6)	16 (12.3)	48 (13.1)				
Exon 11(G/A)	rs12085877 (R439H)	*GG*	122 (74.8)	99 (83.9)	50 (76.9)	149 (81.4)				
		GA	41 (25.2)	19 (16.1)	15 (23.1)	34 (18.6)		NS		NS
		AA	0	0	0	0				
		G	285 (87.4)	217 (91.9)	115 (88.5)	332 (90.7)				
		A	41 (12.6)	19 (8.1)	15 (11.5)	34 (9.3)				
Exon 11(C/T)	rs1782455	*CC*	107 (65.6)	74 (62.7)	41 (63.1)	115 (62.8)				
		CT	54 (33.1)	39 (33.1)	22 (33.8)	61 (33.3)		NS		NS
		TT	2 (1.2)	5 (4.2)	2 (3.1)	7 (3.8)				
		C	268 (82.2)	187 (79.2)	104 (80)	291 (79.5)				
		T	58 (17.8)	49 (20.8)	26 (20)	75 (20.5)				

NS, non-significant; NA, not applicable. Percentages may not sum to 100%, because of rounding errors. Study groups were as follows: the SEP group (cases) was composed of individuals who tested positive for *Schistosoma* eggs, the SELP group (controls) was composed of individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (ELISA), and the SELN group (controls) was composed of individuals who tested negative for *Schistosoma* antigens by ELISA and negative for *Schistosoma* eggs. CI, confidence interval; NS, not significant; OR, odds ratio.

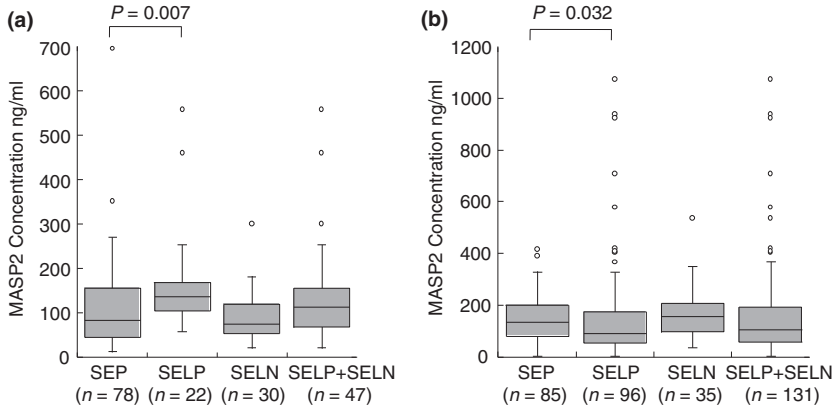

Figure 2 Serum MASP-2 levels (a): in children <12 years of age and (b): in adolescents and adults >12 years of age. Study groups were as follows: the SEP group (cases) was composed of individuals who tested positive for *Schistosoma* eggs, the SELP group (controls) was composed of individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (ELISA), and the SELN group (controls) was composed of individuals who tested negative for Schistosoma antigens by ELISA and negative for *Schistosoma* eggs.

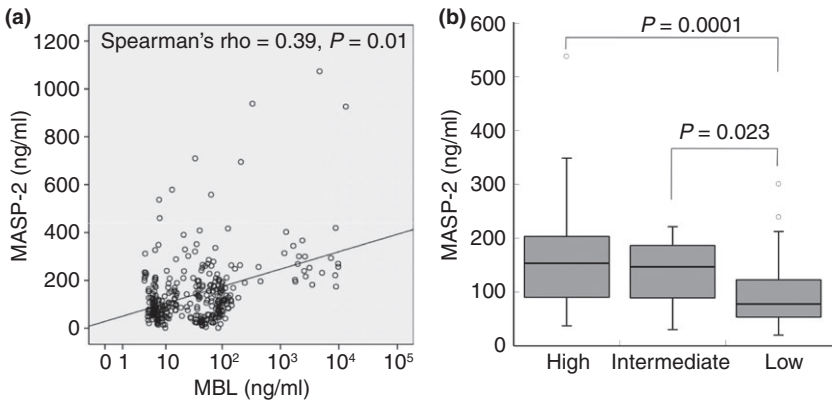

Figure 3 (a). Correlation of serum MBL levels with serum MASP-2 levels. A positive correlation was observed, and *P*-values are given. (b). Distribution of secretor haplotypes in the SELN group (controls). The SELN controls were composed of individuals who tested negative for *Schistosoma* antigens by ELISA and negative for *Schistosoma* eggs.

(SEP) cases nor in exposed (SELP) controls. However, in SELN controls, a significant difference in MASP-2 levels was observed in accordance with secretor haplotypes as MASP-2 serum levels differed with regard to the individuals with high, intermediate or low secretor haplotypes (high [*MASP2*1A, *1B1-h*, **1B2-h*] *vs.* low [*MASP2*1C1-l,*1C2-l,*2A1,*2A2-l* and **2A23-l*] *MASP2* secretor haplotypes $P = 0.0001$; intermediate [*MASP2*2B1-i, *2B2A-i*] vs. low *MASP2* secretor haplotypes; $P = 0.023$; Figure 3b). In SEP cases, the heterozygous genotype p.P126L (*rs56392418CT*) was observed to be associated with reduced circulating MASP-2 serum levels compared to the reference genotype ($P = 0.01$; Figure 4). MASP-2 serum levels were analysed for a possible association with the *MBL2* variants described previously [31]. Neither the *MBL2* alleles nor the *MBL2** secretor haplotypes or genotypes revealed any significant contribution to MASP-2 serum levels.

Discussion

This study investigated the role of functional *MASP2* gene polymorphisms and MASP-2 serum levels in schistosomiasis. The lectin complement pathway acts in the first line of defence that, to a certain degree, can recognise and eliminate many pathogens encountered. Our recent studies have demonstrated that the lectin complement components, namely ficolin-2 [32] and MBL [31], contribute to *S. haematobium* susceptibility.

Despite antigen recognition in the first line of defence, a multitude of factors such as host genetics, worm burden and coinfections with other pathogens contribute to

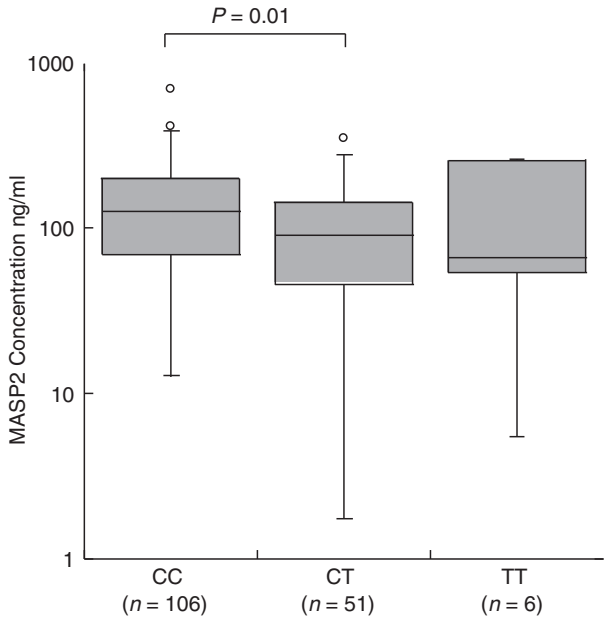

Figure 4 Association of the p.P126L (rs56392418C/T) *MASP2* variant with circulating MASP-2 serum levels in individuals who tested positive for *Schistosoma* eggs (SEP).

S. haematobium survival in human hosts [37, 38]. To establish an active infection, schistosomes need to efficiently evade complement-mediated responses. Schistosomes bind complement proteins on their surfaces to avoid lysis or opsonisation [39, 40]. *In vitro* studies have also confirmed that the complement proteins C1, C4 and C2 are utilised at a larger extent in the presence of *S. mansoni* schistosomula [41], signifying that the high utilisation or strong inhibition of complement proteins is essential for their survival [12]. We hypothesised that the MASP-2 complement protein is equally consumed by *S. haematobium* as is MBL and ficolin-2, providing the opportunity to schistosomes to evade host immune responses. In contrast to our earlier observations of genetic variants in the genes encoding ficolin-2 and MBL, no significant contribution was observed for the functional *MASP2* variants studied. A trend of significance towards an increased risk of schistosomiasis was observed for the homozygous genotype *rs56392418TT* (exon 3, p.P126L). An earlier study has shown that *MASP2* in individuals with the homozygous genotype of *rs56392418TT* in exon 3 (p.126L) was non-functional and unable to cleave C4 and C2 components [25]. Of interest is that, in infected individuals, we observed a significant contribution of the heterozygous genotype p.P126L (*rs56392418CT*) to lower MASP-2 serum levels compared to levels in individuals carrying the reference allele (p.P126, *rs56392418C*). This suggests that the variant p.P126L accounts for the impaired complement activity and lower MASP-2 serum levels.

As shown previously [23, 25], serum MASP-2 levels were associated with distinct *MASP2* genotypes and haplotypes. Our results revealed no significant associations either with genotypes or with haplotypes in the case and the first control subgroup (SELP). Nevertheless, we observed genotype-/haplotype-specific secretor patterns of serum MASP-2 levels in the second control subgroup (SELN), in line with previous findings on the contribution of MASP-2 serum levels associated with distinct secretor haplotypes [23]. No differing distributions were observed of MASP-2 serum levels among the subgroups. Age, however, was a confounding factor. We therefore grouped the study participants based on the common puberty age in the studied population [36]. Differential distributions of MASP-2 serum levels were observed when comparing younger and older children and adults. Younger children (≤12 years) had, during active infection, lower serum MASP-2 levels compared to SELP controls of the same age group, whereas older children and adults (>12 years) with active infections had higher serum MASP-2 levels than individuals of the control group. These results indicate an age-related pattern in the distribution of MASP-2 levels in individuals exposed to and infected with *S. haematobium* and suggest that serum MASP-2 levels may be modified during ongoing infections in an age-dependent manner. Similar age-related patterns in urogenital schistosomiasis have also been identified in studies on other immune components such as myeloid dendritic cells (mDCs) and distinct cytokines, including IL-5 [42–44].

Moreover, the experimental infection of *Schistosoma mansoni* in mice either before or after puberty showed that prepuberty infected mice had a higher number of adult worms, more severe granulomas, higher mortality rates and higher proliferative responses than in post-puberty infected mice [45]. Together, these observations indicate age-related patterns and effects of pubertal development with infection status as observed in our results. In schistosomiasis-endemic areas, a clear association between age and intensity of infection has been well documented. Younger children below the puberty age are generally more heavily infected than older children and adults and are more susceptible to reinfection [35, 45, 46]. In the present study, we observed that *MASP-2* might also contribute to the differential immune response in younger children and adults during urogenital schistosomiasis. Based on our observations, we agree with the hypothesis of 'Puberty and Resistance to Schistosomiasis' proposed by Fulford *et al.*, as it suggests that host age needs to be considered to achieve elimination of

schistosomiasis [46]. However, this hypothesis has not received much attention as no specific mechanisms have been proposed.

There are several limitations in our study: A larger sample size would corroborate the understanding of the contribution of the P126L variant in the studied population. So far, we cannot explain the exact mechanism lying behind our key finding, that is age-related pattern of MASP-2 levels in patent infections. It appears, however, that complement utilisation and low MASP-2 levels are indispensable for *S. haematobium* in establishing a chronic and/or recurrent infection in children. In conclusion, our findings show that high MASP-2 serum levels are associated with relative protection from urogenital schistosomiasis in Nigerian children.

Acknowledgements

We thank Abass Busari, Olaolu Abdulazeez and Adedokun Samuel for technical support. We also thank the staff of the Schistosomiasis Control Unit, Ministry of Health, Abeokuta, and the community members for their participation in the study. We are grateful to Silvelia Grummes for technical help with sequencing.

References

1. WHO. Schistosomiasis. Fact Sheet Number 115. Geneva: World Health Organization, 2014.
2. Hotez PJ, Asojo OA, Adesina AM. Nigeria: "Ground Zero" for the high prevalence neglected tropical diseases. *PLoS Negl Trop Dis* 2012: **6**: e1600.
3. Steinmann P, Keiser J, Bos R *et al.* Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis* 2006: **6**: 411–425.
4. Hotez PJ, Kamath A. Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Negl Trop Dis* 2009: **3**: e412.
5. Ofoezie IE. Patterns of reinfection following praziquantel treatment of urinary schistosomiasis at a period of low transmission. *Acta Trop* 2000: **75**: 123–126.
6. Lustigman S, Prichard RK, Gazzinelli A *et al.* A research agenda for helminth diseases of humans: the problem of helminthiases. *PLoS Negl Trop Dis* 2012: **6**: e1582.
7. Skelly PJ, Alan WR. Making sense of the schistosome surface. *Adv Parasitol* 2006: **63**: 185–284.
8. Peterson NA, Hokke CH, Deelder AM *et al.* Glycotope analysis in miracidia and primary sporocysts of *Schistosoma mansoni*: differential expression during the miracidium-to-sporocyst transformation. *Int J Parasitol* 2009: **39**: 1331–1344.
9. Hayunga EG, Sumner MP. Expression of lectin-binding surface glycoproteins during the development of *Schistosoma mansoni* schistosomula. *J Parasitol* 1986: **72**: 913–920.
10. Rasmussen KR, Kemp WM. *Schistosoma mansoni*: interactions of adult parasites with the complement system. *Parasite Immunol* 1987: **9**: 235–248.
11. Ruppel A, Rother U, Vongerichten H *et al. Schistosoma mansoni*: complement activation in human and rodent sera by living parasites of various developmental stages. *Parasitology* 1983: **87**: 75–86.
12. Klabunde J, Berger J, Jensenius JC *et al. Schistosoma mansoni*: adhesion of mannan-binding lectin to surface glycoproteins of cercariae and adult worms. *Exp Parasitol* 2000: **95**: 231–239.
13. Assaf A, Hoang TV, Faik I *et al.* Genetic evidence of functional ficolin-2 haplotype as susceptibility factor in cutaneous leishmaniasis. *PLoS ONE* 2012: **7**: e34113.
14. Hoang TV, Toan NL, Song lH *et al.* Ficolin-2 levels and FCN2 haplotypes influence hepatitis B infection outcome in Vietnamese patients. *PLoS ONE* 2011: **6**: e28113.
15. Jha AN, Sundaravadivel P, Singh VK *et al.* MBL2 variations and malaria susceptibility in Indian populations. *Infect Immun* 2014: **82**: 52–61.
16. Luz PR, Boldt AB, Grisbach C *et al.* Association of L-ficolin levels and FCN2 genotypes with chronic Chagas disease. *PLoS ONE* 2013: **8**: e60237.
17. Velavan TP, Boldt AB, Tomiuk J *et al.* Variant alleles of the mannose binding lectin 2 gene (MBL2) confer heterozygote advantage within Crohn's families. *Scand J Gastroenterol* 2010: **45**: 1129–1130.
18. Holmskov U, Thiel S, Jensenius JC. Collections and ficolins: humoral lectins of the innate immune defense. *Annu Rev Immunol* 2003: **21**: 547–578.
19. Wallis R, Dodds AW, Mitchell DA *et al.* Molecular interactions between MASP-2, C4, and C2 and their activation fragments leading to complement activation via the lectin pathway. *J Biol Chem* 2007: **282**: 7844–7851.
20. Degn SE, Jensen L, Hansen AG *et al.* Mannan-binding lectin-associated serine protease (MASP)-1 is crucial for lectin pathway activation in human serum, whereas neither MASP-1 nor MASP-3 is required for alternative pathway function. *J Immunol* 2012: **189**: 3957–3969.
21. Rossi V, Cseh S, Bally I *et al.* Substrate specificities of recombinant mannan-binding lectin-associated serine proteases-1 and -2. *J Biol Chem* 2001: **276**: 40880–40887.
22. Stover C, Endo Y, Takahashi M *et al.* The human gene for mannan-binding lectin-associated serine protease-2 (MASP-2), the effector component of the lectin route of complement activation, is part of a tightly linked gene cluster on chromosome 1p36.2-3. *Genes Immun* 2001: **2**: 119–127.
23. Boldt AB, Grisbach C, Steffensen R *et al.* Multiplex sequence-specific polymerase chain reaction reveals new MASP2 haplotypes associated with MASP-2 and MAp19 serum levels. *Hum Immunol* 2011a: **72**: 753–760.
24. Thiel S, Kolev M, Degn S *et al.* Polymorphisms in mannan-binding lectin (MBL)-associated serine protease 2 affect stability, binding to MBL, and enzymatic activity. *J Immunol* 2009: **182**: 2939–2947.

25. Thiel S, Steffensen R, Christensen IJ *et al.* Deficiency of mannan-binding lectin associated serine protease-2 due to missense polymorphisms. *Genes Immun* 2007: **8**: 154–163.
26. Boldt AB, Goeldner I, Stahlke ER *et al.* Leprosy association with low MASP-2 levels generated by MASP2 haplotypes and polymorphisms flanking MAp19 exon 5. *PLoS ONE* 2013: **8**: e69054.
27. Boldt AB, Luz PR, Messias-Reason IJ. MASP2 haplotypes are associated with high risk of cardiomyopathy in chronic Chagas disease. *Clin Immunol* 2011c: **140**: 63–70.
28. Holmberg V, Onkamo P, Lahtela E *et al.* Mutations of complement lectin pathway genes MBL2 and MASP2 associated with placental malaria. *Malar J* 2012: **11**: 61.
29. Schafranski MD, Pereira FL, Scherner D *et al.* Functional MASP2 gene polymorphism in patients with history of rheumatic fever. *Hum Immunol* 2008: **69**: 41–44.
30. Tulio S, Faucz FR, Werneck RI *et al.* MASP2 gene polymorphism is associated with susceptibility to hepatitis C virus infection. *Hum Immunol* 2011: **72**: 912–915.
31. Antony JS, Ojurongbe O, Van TH *et al.* Mannose-binding lectin and susceptibility to schistosomiasis. *J Infect Dis* 2013: **207**: 1675–1683.
32. Ouf EA, Ojurongbe O, Akindele AA *et al.* Ficolin-2 levels and FCN2 genetic polymorphisms as a susceptibility factor in schistosomiasis. *J Infect Dis* 2012: **206**: 562–570.
33. Boldt AB, Grisbach C, Steffensen R *et al.* Multiplex sequence-specific polymerase chain reaction reveals new MASP2 haplotypes associated with MASP-2 and MAp19 serum levels. *Hum Immunol* 2011b: **72**: 753–760.
34. Knobloch J, Delgado E. Immunodiagnosis of cysticercosis: standardization of ELISA and its application to field conditions. *Trop Med Parasitol* 1985: **36**: 157–159.
35. Dunne DW, Butterworth AE, Fulford AJ *et al.* Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* 1992: **22**: 1483–1494.
36. Fakeye O, Fagbule D. Age and anthropometric status of Nigerian girls at puberty: implication for the introduction of sex education into secondary schools. *West Afr J Med* 1990: **9**: 226–231.
37. Loukas A, Jones MK, King LT *et al.* Receptor for Fc on the surfaces of schistosomes. *Infect Immun* 2001: **69**: 3646–3651.
38. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. *Nat Rev Immunol* 2002: **2**: 499–511.
39. Santoro F, Ouaissi MA, Pestel J *et al.* Interaction between *Schistosoma mansoni* and the complement system: binding of C1q to schistosomula. *J Immunol* 1980: **124**: 2886–2891.
40. Tarleton RL, Kemp WM. Demonstration of IgG-Fc and C3 receptors on adult *Schistosoma mansoni*. *J Immunol* 1981: **126**: 379–384.
41. Ouaissi MA, Auriault C, Santoro F *et al.* Interaction between *Schistosoma mansoni* and the complement system: role of IgG Fc peptides in the activation of the classical pathway by schistosomula. *J Immunol* 1981: **127**: 1556–1559.
42. Milner T, Reilly L, Nausch N *et al.* Circulating cytokine levels and antibody responses to human *Schistosoma haematobium*: IL-5 and IL-10 levels depend upon age and infection status. *Parasite Immunol* 2010: **32**: 710–721.
43. Mutapi F, Winborn G, Midzi N *et al.* Cytokine responses to *Schistosoma haematobium* in a Zimbabwean population: contrasting profiles for IFN-gamma, IL-4, IL-5 and IL-10 with age. *BMC Infect Dis* 2007: **7**: 139.
44. Nausch N, Louis D, Lantz O *et al.* Age-related patterns in human myeloid dendritic cell populations in people exposed to *Schistosoma haematobium* infection. *PLoS Negl Trop Dis* 2012: **6**: e1824.
45. Yole DS, Gikuru SK, Wango EO *et al.* Influence of age of mice on the susceptibility to murine schistosomiasis infection. *Afr. J. Health Sci.* 2006: **13**: 47–54.
46. Fulford AJ, Webster M, Ouma JH *et al.* Puberty and age-related changes in susceptibility to schistosome infection. *Parasitol Today* 1998: **14**: 23–26.

Corresponding Author Thirumalaisamy P. Velavan, Institute of Tropical Medicine, University of Tübingen, Wilhelmstrasse 27, 72074 Tübingen, Germany. Tel.: +49-7071-2985981; Fax: +49-7071-294684; E-mail: velavan@medizin.uni-tuebingen.de

Parasitology International 64 (2015) 591–596

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Mannose-binding Lectin (MBL) as a susceptible host factor influencing Indian Visceral Leishmaniasis

Anshuman Mishra [a,1], Justin S. Antony [b,1], Prabhanjan Gai [b], Pandarisamy Sundaravadivel [a], Tong Hoang van [b], Aditya Nath Jha [a], Lalji Singh [a,c], Thirumalaisamy P. Velavan [b,d,2,*], Kumarasamy Thangaraj [a,2]

[a] CSIR — Center for Cellular and Molecular Biology, Hyderabad, India
[b] Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany
[c] Banaras Hindu University, Varanasi, India
[d] Fondation Congolaise pour la Recherche Medicale, Brazzaville, Congo

ARTICLE INFO

Article history:
Received 16 February 2015
Received in revised form 31 July 2015
Accepted 7 August 2015
Available online 19 August 2015

Keywords:
Visceral Leishmaniasis
Leishmania donovani
MBL2
MBL serum level
India

ABSTRACT

Visceral Leishmaniasis (VL), caused by *Leishmania donovani* is endemic in the Indian sub-continent. Mannose-binding Lectin (MBL) is a complement lectin protein that binds to the surface of *Leishmania* promastigotes and results in activation of the complement lectin cascade. We utilized samples of 218 VL patients and 215 healthy controls from an Indian population. *MBL2* functional variants were genotyped and the circulating MBL serum levels were measured. MBL serum levels were elevated in patients compared to the healthy controls (adjusted $P = 0.007$). The *MBL2* promoter variants −78C/T and +4P/Q were significantly associated with relative protection to VL (−78C/T, OR = 0.7, 95% CI = 0.5–0.96, adjusted $P = 0.026$ and +4P/Q, OR = 0.66, 95% CI = 0.48–0.9, adjusted $P = 0.012$). *MBL2*LYQA* haplotypes occurred frequently among controls (OR = 0.69, 95% CI = 0.5–0.97, adjusted $P = 0.034$). MBL recognizes *Leishmania* and plays a relative role in establishing *L. donovani* infection and subsequent disease progression. In conclusion, *MBL2* functional variants were associated with VL.

1. Introduction

Leishmaniasis is a neglected tropical disease, caused by parasites of the genus *Leishmania* that inhabits host macrophages. Leishmania infection is transmitted by an infective bite of a female phlebotomine sand fly. Leishmaniasis is endemic in tropical regions including the Indian sub-continent, Africa and in South America and accounts for approximately 1.3 million new cases every year [1]. Four distinct clinical features of Leishmaniasis include, Cutaneous Leishmaniasis (CL), Muco-cutaneous Leishmaniasis (MCL), Visceral Leishmaniasis (VL or Kala-azar) and Post-Kala-azar Dermal Leishmaniasis (PKDL) [2]. Visceral Leishmaniasis (VL) is the severest form, caused by an intracellular pathogen *Leishmania donovani* and is fatal if left untreated [3]. VL still remains a global health burden with 400,000 new cases and 40,000 deaths reported annually [4,5]. VL accounts for more than 2 million disability-adjusted life years (DALYs) lost [6] and approx. 90% of these cases occur in Bangladesh, India, Nepal, Sudan, Ethiopia and Brazil [2]. VL is considered as a major health problem in the Indian sub-continent with an estimated 150 million people living at risk and patients from this region share 67% of the global disease burden [7]. In India, VL is endemic in Central-East India (Bihar, West Bengal and Eastern part of Uttar Pradesh) and accounts for more than 90% of all Indian VL [8].

Leishmania is a Trypanosomatid protozoan parasite, with two different developmental stages namely the promastigotes and amastigotes [9]. In both developmental stages, the parasites are enveloped with mannose-containing lipophosphoglycan (LPG) and mannose glycoinositol-phospholipids [10]. These glycoconjugates act as pathogen associated molecular patterns (PAMPs) and are recognized by the lectin complement components such as the Mannose-binding Lectin (MBL). Earlier studies have shown that, *Leishmania major*, *Leishmania mexicana*, *Leishmania chagasi* and *Leishmania braziliensis* were recognized by the carbohydrate recognition domain (CRD) of the MBL protein [10–12]. MBL binds to the surface of the promastigotes and activates complement lectin cascade, thereby promastigotes are phagocytosed by the macrophages.

MBL is a circulating serum protein and a pathogen recognition receptor (PRR) which binds to PAMPs, leading to the activation of the lectin complement cascade [13]. The human MBL encoded by *MBL2* gene is located on chromosomes 10q11.2–q21. MBL has higher affinity to microbial polysaccharides and/or their glycoconjugates, which are rich in mannose and fucose sugars. MBL was shown earlier to bind cell surfaces of bacteria, fungi, protozoa and viruses and acts as an acute-phase plasma protein (APP) during infection and inflammation [14,15]. When infection progresses, MBL serum levels increase to 1.5–3.0 folds [16] and

* Corresponding author at: Institute of Tropical Medicine, University of Tübingen, Wilhelmstrasse 27, 72074 Tübingen, Germany.
E-mail address: velavan@medizin.uni-tuebingen.de (T.P. Velavan).
[1] Equal contributions.
[2] Shared senior authors.

http://dx.doi.org/10.1016/j.parint.2015.08.003
1383-5769/

act as a pro-inflammatory marker [17]. The carbohydrate recognition domain (CRD) of the MBL recognizes the parasite epitopes [18]. Functional *MBL2* variants in the promoter region and in exon 1 were shown to modulate the expression and oligomerization of the protein, respectively [19]. Three *MBL2* promoter variants at positions −550(H/L), −221(X/Y) and +4(P/Q) modulate the MBL transcriptional activity [20,21]. In addition, three non-synonymous substitutions in exon 1 at codons 52 (*MBL2*D*), 54 (*MBL2*B*) and 57 (*MBL2*C*) disrupt the oligomerization of the MBL protein to the pathogen, and thus impede activation of the lectin complement cascade. Seven common secretor *MBL2* haplotypes were reported. The haplotypes *MBL2*HYPA*, *LYPA* and *LYQA* are associated with high expression of MBL, while the haplotypes *MBL2*LYQC*, *LXPA*, *HYPD* and *LYPB* are associated with low expression of MBL [18]. Lectin complement proteins have been associated with many infectious diseases including intra- and extracellular infections [22–25]. Our earlier study has shown that, another analogous lectin complement protein ficolin-2 was associated with occurrence of VL [26].

Earlier studies on Brazilian patients have shown that MBL plays a significant role in VL caused by *L. chagasi* [11,27]. In this study, we aim to investigate the possible association of circulating MBL levels and *MBL2* functional variants during VL caused by the intracellular pathogen *L. donovani*.

2. Material and methods

2.1. Ethical approval

Informed written consent was provided either by the participating individual or from the parents, if patient was less than 18 years of age. The study was approved by the Institutional Ethical Committee (IEC) of Center for Cellular and Molecular Biology (CSIR), Hyderabad, India and prior permission was obtained from the district government hospitals.

2.2. Study design and sample collection

This cross-sectional, case–control study was conducted in an endemic region with high incidence of VL. All patients and controls were recruited through multiple field visits from villages located within a radius of approx. 120 km from the city of Muzaffarpur in the Bihar state. Previous epidemiological studies have indicated that, Bihar state is a hot spot for VL with an average annual incidence of 2.49/1000 individuals [28,29]. The sample size was calculated prior to recruitment using the Open Epi platform (http://www.openepi.com/) based on the incidence rate and the risk of VL in the study area. A total of 443 unrelated subjects (218 cases and 225 healthy controls) were recruited. The mean age of VL patients and healthy controls was 28.7 ± 16.7 and 35.3 ± 16.2, respectively ($P = 0.001$). No significant difference in male/female ratio was observed between cases (125:95) and controls (122:93). The cases were determined based on the clinical features of VL in medical records issued by government hospitals in the study region. Typical clinical features of the patients included fever with rigors and chills and splenomegaly. Patients and controls were tested with the rk39 leishmanin antigen by nitrocellulose dipstick tests (InBios International, Seattle, USA). The control subjects were free of any relevant infectious disease. Pregnant women, patients with other infections, healthy controls with a family history of VL and relatives of cases were excluded from the study. Approximately 5 ml of venous blood was collected from each study participant who presented at the time of diagnosis at a referral center The samples were immediately transported to the lab and were stored at −20 °C until use.

2.3. MBL serum level measurement

MBL serum levels were measured in VL patients (n = 101) and healthy controls (n = 60) using a human MBL ELISA kit following the manufacturer's instructions (Hycult Biotech, Uden, The Netherlands). The detection limit of the assay was 0.4 ng/ml.

2.4. MBL2 genotyping

Genomic DNA was extracted from peripheral blood leucocytes using a QIAamp DNA mini blood kit (Qiagen, Hilden, Germany). *MBL2* promoter polymorphisms at −550G/C, −6 bp deletion −338 to −332, −221G/C and 5′UTR +4C/T and exon 1 polymorphisms at codons 52C/T, 54G/A and 57G/A were amplified by polymerase chain reaction using primer pairs spanning the promoter region to exon 1. The primers employed were: PromF: 5′-GCCAGAAAGTAGAGAGGTATTTAGCAC-3′, internal primer Exon1F: 5′-CAGGTGTCTAGGCACAGATGAACC-3′ and Exon1R: 5′-CCAACACGTACCTGGTTCCC-3′. In brief: 20 ng of genomic DNA was amplified in a 10 μl volume of reaction mixture containing 1× PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl, 1.5 mM of $MgCl_2$), 0.125 mM of dNTPs, 0.25 mM of each primer and 1 U Taq DNA polymerase (TaKaRa, Shiga, Japan). Thermal cycling parameters for amplification of both exon 1 and promoter regions were: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C denaturation, 30 s at 68 °C annealing temperature, 1 min 30 s at 72 °C extension, followed by a final extension of 2 min at 72 °C. The amplified PCR fragments were run on a 1.5% agarose gel and were visualized using a UV transilluminator. PCR products were cleaned up using Exo-SAP-IT (USB-Affymetrix, Santa Clara, USA) and 1 μl of the purified product was directly used as templates for sequencing using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in an automated sequencer (ABI Prism 3730XL Genetic Analyzer, Applied Biosystems). The resulting DNA sequences were aligned using AutoAssembler software (Applied Biosystems, Foster City, CA, USA) and were reconfirmed visually from their electropherograms.

2.5. Statistical analysis

Data were analyzed using the STATA software (Intercooled STATA, STATA Corp., College Station, TX, USA) and the level of significance was set to a P value of <0.05 in all comparisons. Allele, genotype and haplotype frequencies were calculated by simple gene counting and by expectation–maximum (EM) algorithm and the deviations from the Hardy–Weinberg equilibrium were tested using the random-permutation procedure as implemented in the Arlequin v.3.5.1.2 software (http://lgb.unige.ch/arlequin). The linkage disequilibrium (LD) analysis was performed using Haploview v.3.2 (http://broadinstitute.org/haploview). Binary logistic regression was performed to adjust for the confounding effects such as the age, ethnicity and gender. Multivariate analysis was performed to analyze the differences in serum MBL levels between cases and controls adjusted for age, ethnicity and gender. Kruskal–Wallis or Wilcoxon Mann–Whitney rank sum tests were applied to analyze the association of serum MBL levels with *MBL2* variants and haplotypes by using the SPSS v.19 software (IBM Corp. USA).

3. Results

3.1. MBL serum levels and Visceral Leishmaniasis

The MBL serum levels in the studied healthy individuals varied from 0.49 to 911 ng/ml with a median of 78.6 ng/ml while the MBL serum levels in the VL cases varied from 0.47 to 833.3 ng/ml with median levels of 155.1 ng/ml. We observed increased MBL serum levels in the VL cases compared to those in the healthy controls (adjusted $P = 0.007$) (Fig. 1).

3.2. MBL2 variants and Visceral Leishmaniasis

The genotype frequencies for all the analyzed *MBL2* functional SNPs in each respective group were in the Hardy–Weinberg equilibrium except for variant rs10556764 (−338 to −332 6 bp deletion) in VL

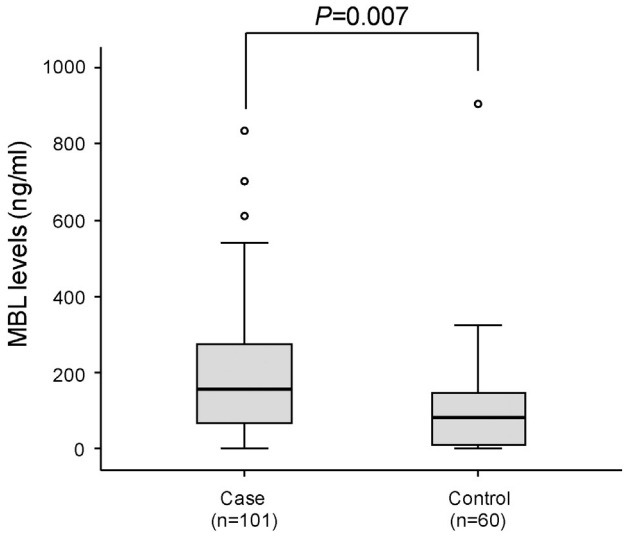

Fig. 1. Distribution of MBL serum levels in Visceral Leishmaniasis patients and healthy controls. *P* values were calculated by using multivariate analysis adjusted for age, sex and ethnicity.

cases therefore this variant was excluded from further association analysis. Linkage disequilibrium pattern of studied *MBL2* variants in VL cases and controls are shown in Fig. 2. The Linkage Disequilibrium (LD) pattern revealed that the promoter variants −78C/T and +4P/Q are in strong LD both in VL cases and controls. The *MBL2* minor allele −78T in the promoter region was observed to be significantly higher in healthy controls compared to that in the VL cases after adjusting for age, sex and ethnicity (OR = 0.7, 95% CI = 0.5–0.96, adjusted P = 0.026). The frequency of minor allele *+4Q* at the transcription start site was observed to be significantly higher in healthy controls than that of patients after adjusting for age, sex and ethnicity (OR = 0.66, 95% CI = 0.48–0.9, adjusted P = 0.012). These results suggest that *MBL2* variants −78C/T and +4P/Q were significantly associated with relative protection against VL (Table 1). A similar effect was also observed with the dominant genetic model. In addition, other investigated *MBL2* functional variants in the promoter region [(−550H/L), (−221X/Y)] and exon 1 region [codons 52 (*MBL2*D*), 54 (*MBL2*B*) and 57 (*MBL2*C*)] were not significantly associated with VL (data not shown).

3.3. MBL2 haplotype and Visceral Leishmaniasis

The distribution of reconstructed *MBL2* haplotypes is summarized in Table 2. In total, sixteen secretor haplotypes were observed in this study. Five main haplotypes *MBL2*LYPA*, *MBL2*HYPA*, *MBL2*LXPA*, *MBL2*LYQA* and *MBL2*LYPB* were found at higher frequencies (>10%). However, only the *MBL2*LYQA* haplotype was observed more frequently in healthy controls than that in VL patients after adjusting for age, sex and ethnicity (OR = 0.69, 95% CI = 0.5–0.97, adjusted P = 0.034). The other haplotypes remained insignificant when compared between VL cases and control group (Table 2). The contribution of either high secretor (*LYPA+LYQA+HYPA*) or low secretor (*LYQC+LXPA+HYPD+LYPB*) haplotypes revealed no significance (Table 2). In addition, investigation of *MBL2* diplotypes (*YA/YA*, *YA/YO*, *XA/YA*, *XA/YO*, *YO/YO* and *XA/XA*) also revealed no significant association with VL (data not shown).

3.4. Association of MBL2 variants with MBL serum levels

The association of −78C/T and +4P/Q variants with circulating MBL serum levels was analyzed. MBL serum levels were segregated according to different genotypes of −78C/T and +4P/Q variants in VL cases and healthy controls. The results indicated that the minor alleles *−78T* and *+4Q* contribute to increased MBL serum levels, whereas the major alleles *−78C* and *+4P* contribute to decreased MBL serum levels in healthy controls (P = 0.024 and 0.033, respectively) (Fig. 3). Also a gene dose dependent effect on the distribution of serum MBL levels was observed. However, the −78C/T and +4P/Q variants did not show any significant contribution to MBL serum levels in VL patients.

4. Discussion

The clinical progression of VL is modulated by multitude of factors such as host genetics, immunological factors and pathogenesis of *Leishmania* spp. [30]. Geographical heterogeneity and host genetic variations in *HLA-DRB1*, *HLA-DQA1*, *HLA* class II region, *SLC11A1* and *DLL1* were shown to modulate VL susceptibility in Indian patients [31–33]. Human and animal models have shown that host genetic factors play

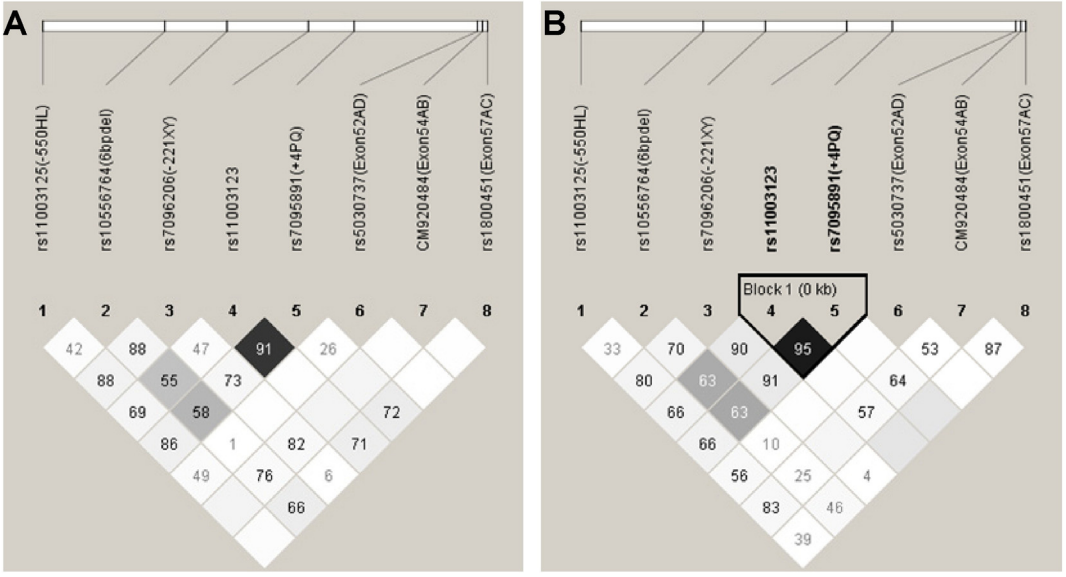

Fig. 2. Linkage disequilibrium pattern of studied SNPs. (A): Linkage disequilibrium pattern of studied *MBL2* variants in Visceral Leishmaniasis patients and (B): Linkage disequilibrium pattern of studied *MBL2* variants in healthy controls. Numbers indicate the D′ value expressed as percentile. Open squares indicate the high degree of LD (D′ = 1). The dense shading indicates the r^2 value.

Table 1
Distribution of *MBL2* genotypes and alleles in Visceral Leishmaniasis patients and healthy controls.

SNP position	Cases	Controls	Cases vs. controls	
	n = 218 (%)	n = 215 (%)	OR (95% CI)	*P* value[a]
rs11003123 (−78C/T)				
CC	131 (60.1)	108 (50.2)		
CT	73 (33.5)	85 (39.6)		
TT	14 (6.4)	22 (10.2)		
Allele				
C	335 (76.8)	301 (70)	1	Reference
T	101 (23.2)	129 (30)	0.7 (0.5–0.96)	0.026
Dominant				
CC	131 (60.1)	108 (50.2)	1	Reference
CT+TT	78 (39.9)	107 (49.8)	0.66 (0.45–0.99)	0.044
Recessive				
CC+CT	104 (93.6)	193 (89.8)	1	Reference
TT	14 (6.4)	22 (10.2)	NA	NS
rs7095891 (+4C/T)				
CC (PP)	134 (61.5)	110 (51.2)		
CT (PQ)	72 (33.0)	83 (38.6)		
TT (QQ)	12 (5.5)	22 (10.2)		
Allele				
C (P)	340 (78)	303 (70.5)	1	Reference
T (Q)	96 (22)	127 (29.5)	0.66 (0.48–0.9)	0.012
Dominant				
CC (PP)	134 (61.5)	110 (51.2)	1	Reference
CT(PQ)+TT(QQ)	84 (38.5)	105 (48.8)	0.65 (0.44–0.97)	0.037
Recessive				
CC(PP)+CT(PQ)	206 (94.5)	193 (89.8)	1	Reference
TT (QQ)	12 (5.5)	22 (10.2)	NA	NS

Genotype frequencies of *MBL2* SNPs were tested for the Hardy–Weinberg equilibrium in each group using Arlequin. No significant SNP-specific deviation was observed ($P > 0.05$).
NS: Not significant; NA: not applicable.
[a] *P* values were calculated by logistic regression adjusted for age, gender and ethnicity.

a vital part in determining the severity of VL and subsequent progression [34]. The MBL is one such protein that can recognize the invaded metacyclic promastigotes and VL outcome [35]. In this study, we investigated the *MBL2* functional polymorphisms and respective MBL serum levels with VL susceptibility in an Indian population and MBL serum levels and *MBL2* variants (−78C/T and +4P/Q) were significantly associated with VL.

Table 2
Distribution of *MBL2* haplotypes in Visceral Leishmaniasis patients and healthy controls.

MBL2 haplotype	Cases	Controls	Cases vs. controls	
	n = 436 (%)	n = 430 (%)	OR (95% CI)	*P* value[a]
LYPA	101 (23.2)	78 (18.1)		NS
HYPA	87 (20)	79 (18.4)		NS
LXPA	81 (18.6)	82 (19.1)		NS
LYQA	77 (17.7)	99 (23)	0.69 (0.5–0.97)	0.034
LYPB	57 (13.1)	51 (11.9)		NS
LYQC	14 (3.2)	17 (4)		NS
HYPD	7 (1.6)	7 (1.6)		NS
HYQA	4 (0.9)	6 (1.4)		NS
LYPD	3 (0.7)	3 (0.7)		NS
HYPB	1 (0.2)	1 (0.2)		NS
LYPC	3 (0.7)	0		NA
HXQA	1 (0.2)	0		NA
LYQB	0	3 (0.7)		NA
HXPA	0	2 (0.5)		NA
LXQA	0	1 (0.2)		NA
HYQC	0	1 (0.2)		NA
High expression of MBL				
LYPA+LYQA+HYPA	265 (60.8)	256 (59.5)		Reference
Low expression of MBL				
LYQC+LXPA+HYPD+LYPB	159 (36.5)	157 (36.5)		NS

NS: not significant; NA: not applicable.
[a] *P* values were calculated by logistic regression adjusted for age, gender and ethnicity.

The MBL serum levels were observed to be high in the VL patients than those in the controls. These results support previous findings showing high MBL levels during *L. donovani* infection [11,27]. The elevated MBL levels may be due to the acute-phase reactions during distinct infections [16]. Moreover, the MBL serum levels observed in this study were in accordance with another study on pulmonary tuberculosis, which confirmed higher MBL levels during intracellular infections [36]. Congenital MBL deficiency per se increases the susceptibility to many infections [37]. In addition, a recent study has shown that, complement receptor type 1, expressed on macrophages binds to MBL and enhances the phagocytosis of intra-cellular pathogens [38]. Therefore, high MBL levels may aid in establishing intra-cellular infections. However, decreased MBL proteins will result in fewer parasites being phagocytosed by macrophages, their preferred environment and thus result in a balanced immune response [36].

In our investigations, we observed a promoter polymorphism −78C/T as a possible locus associated with VL occurrence. The minor allele *−78T* was significantly associated with decreased susceptibility to VL. The contribution of this studied *MBL2* −78C/T with other diseases is limited. This particular *MBL2* −78C/T variant has been reported in Han Chinese and Gabonese populations [39,40]. In the HapMap database, the investigated variant −78C/T was widely distributed across all ethnicities and was observed to be in high LD with another variant +4P/Q in the 5′UTR region of exon 1 especially in the Han Chinese population. However, no significant associations with leprosy were reported in the Han Chinese population [39]. Also in this study, the investigated variants +4P/Q and −78C/T were in strong LD. The recruited individuals are from the Bihar state, a geographical location endemic for VL with different ethnicities. The influence of confounding factors such as age, ethnicity and gender was considered in the analyses in order to identify independent effects of *MBL2* variants on VL outcome.

The minor allele *+4Q* was earlier reported to be associated with high MBL expression in different geographically isolated populations [41,42]. An earlier study has reported that, the heterozygous genotypes (*+4PQ*), is a risk factor for schistosomiasis in an African population [43]. In our investigations, no significant associations were observed for the promoter variant −221X/Y. However, the heterozygous genotype *−221XY* conferred a protective role against VL in a studied Morocco population [44]. This particular study on Moroccan VL patients has analyzed −221X/Y genotypes from an asymptomatic group, and the analyzed genotypes were not in the Hardy–Weinberg equilibrium therefore these results are void. The studied exon 1 variants at codons 52, 54 and 57 were not associated with the VL. *MBL2* haplotype analysis revealed that the *MBL2*LYQA* haplotype was significantly higher in healthy controls compared to that in the VL patients indicating that individuals with this haplotype have reduced risk for VL. The *MBL2*LYQA* haplotype is associated with higher MBL expression and reduced risk in severe malaria patients [24]. The *MBL2*LYPA* haplotype was a risk factor in a study on Brazilian patients with leprosy caused by another intracellular pathogen [45]. These results indicate that the role of *MBL2* variants in different intracellular infections likely depends on different geographical settings. The *MBL2* genotypes and haplotypes were associated with MBL protein expression in different world populations [14,43,46].

In the current study, the *MBL2* +4P/Q genotypes were also associated with serum MBL levels in healthy individuals, but not in VL patients. The infection phenotype may possibly contribute to higher MBL levels in VL patients. The *Leishmania* spp. promastigotes have a significant role both in classical [47,48] and alternative pathways [49], as lysis of promastigotes is mediated via an antibody-independent mechanism [47–49]. Yet another study has shown that the binding of MBL to the *Leishmania* surface activates the alternative pathway [12]. In addition, MBL-opsonized *L. chagasi* promastigotes can induce TNF-α and IL6 production by monocytes. These observations support the mechanism that MBL modulates inflammatory cytokine production, such as TNF-α and IL6 [11].

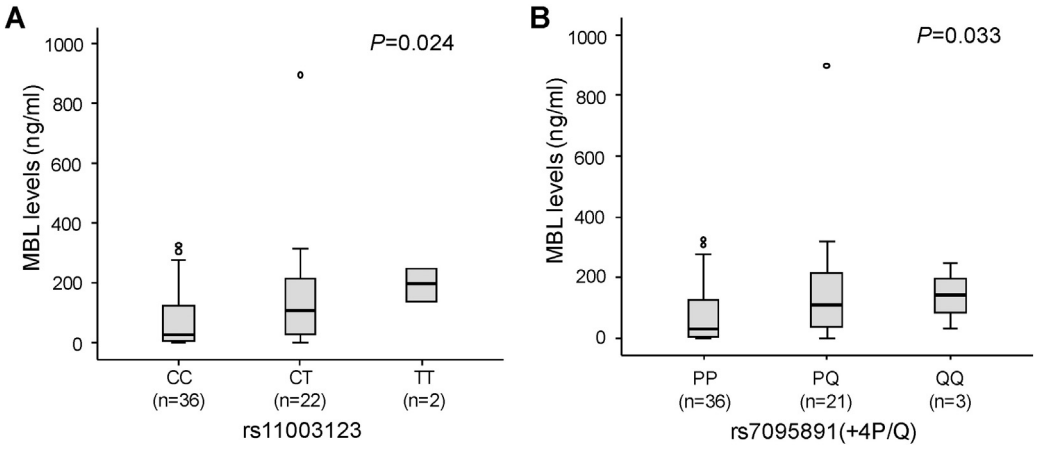

Fig. 3. Distribution of MBL serum levels for different genotypes of −78C/T variant (A) and +4P/Q variant (B) in control individuals. *P* values were calculated by using the Kruskal–Wallis test.

In conclusion, *MBL2* promoter variants are significantly associated with VL caused by *L. donovani*. MBL serum levels were elevated in VL patients and increased MBL levels aid in establishing intra-cellular infections and thus modulated the VL outcome.

Conflict of interests

The authors have no conflict of interests to declare.

Acknowledgments

We thank all the staff of the District Government hospitals in the study area and the community members for their participation in the study. Anshuman Mishra was supported by Department of Biotechnology (www.dbtindia.nic.in), Research Associate Programme, Government of India. Kumarasamy Thangaraj was supported by Council for Scientific and Industrial Research (www.csir.res.in) Network project (Genesis-BSC0121).

References

[1] WHO, Leishmaniasis, Fact Sheet No. 3752013.
[2] F. Chappuis, S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R.W. Peeling, et al., Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat. Rev. Microbiol. 5 (2007) 873–882.
[3] P. Desjeux, Leishmaniasis. Public health aspects and control, Clin. Dermatol. 14 (1996) 417–423.
[4] P. Desjeux, Leishmaniasis: current situation and new perspectives, Comp. Immunol. Microbiol. Infect. Dis. 27 (2004) 305–318.
[5] P.D. Ready, Epidemiology of visceral leishmaniasis, Clin. Epidemiol. 6 (2014) 147–154.
[6] C.D. Mathers, M. Ezzati, A.D. Lopez, Measuring the burden of neglected tropical diseases: the global burden of disease framework, PLoS Negl. Trop. Dis. 1 (2007), e114.
[7] P.J. Hotez, J.H. Remme, P. Buss, G. Alleyne, C. Morel, J.G. Breman, Combating tropical infectious diseases: report of the Disease Control Priorities in Developing Countries Project, Clin. Infect. Dis. 38 (2004) 871–878.
[8] D. Bora, Epidemiology of visceral leishmaniasis in India, Natl Med. J. India 12 (1999) 62–68.
[9] K.P. Chang, D.M. Dwyer, Multiplication of a human parasite (*Leishmania donovani*) in phagolysosomes of hamster macrophages in vitro, Science 193 (1976) 678–680.
[10] P.J. Green, T. Feizi, M.S. Stoll, S. Thiel, A. Prescott, M.J. McConville, Recognition of the major cell surface glycoconjugates of *Leishmania* parasites by the human serum mannan-binding protein, Mol. Biochem. Parasitol. 66 (1994) 319–328.
[11] I.K. Santos, C.H. Costa, H. Krieger, M.F. Feitosa, D. Zurakowski, B. Fardin, et al., Mannan-binding lectin enhances susceptibility to visceral leishmaniasis, Infect. Immun. 69 (2001) 5212–5215.
[12] A.R. Ambrosio, I.J. de Messias-Reason, *Leishmania* (Viannia) *braziliensis*: interaction of mannose-binding lectin with surface glycoconjugates and complement activation. An antibody-independent defence mechanism, Parasite Immunol. 27 (2005) 333–340.
[13] K. Takahashi, R.A. Ezekowitz, The role of the mannose-binding lectin in innate immunity, Clin. Infect. Dis. 41 (Suppl. 7) (2005) S440–S444.
[14] D.L. Worthley, P.G. Bardy, C.G. Mullighan, Mannose-binding lectin: biology and clinical implications, Intern. Med. J. 35 (2005) 548–555.
[15] J.S. Antony, O. Ojurongbe, C.G. Meyer, K. Thangaraj, A. Mishra, P.G. Kremsner, et al., Correlation of interleukin-6 levels and lectins during *Schistosoma haematobium* infection, Cytokine (2015) http://dx.doi.org/10.1016/j.cyto.2015.04.019.
[16] S. Thiel, U. Holmskov, L. Hviid, S.B. Laursen, J.C. Jensenius, The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response, Clin. Exp. Immunol. 90 (1992) 31–35.
[17] M.D. Schafranski, A. Stier, R. Nisihara, I.J. Messias-Reason, Significantly increased levels of mannose-binding lectin (MBL) in rheumatic heart disease: a beneficial role for MBL deficiency, Clin. Exp. Immunol. 138 (2004) 521–525.
[18] R.M. Dommett, N. Klein, M.W. Turner, Mannose-binding lectin in innate immunity: past, present and future, Tissue Antigens 68 (2006) 193–209.
[19] P. Garred, F. Larsen, J. Seyfarth, R. Fujita, H.O. Madsen, Mannose-binding lectin and its genetic variants, Genes Immun. 7 (2006) 85–94.
[20] D.P. Eisen, R.M. Minchinton, Impact of mannose-binding lectin on susceptibility to infectious diseases, Clin. Infect. Dis. 37 (2003) 1496–1505.
[21] D.P. Eisen, M.M. Dean, M.A. Boermeester, K.J. Fidler, A.C. Gordon, G. Kronborg, et al., Low serum mannose-binding lectin level increases the risk of death due to pneumococcal infection, Clin. Infect. Dis. 47 (2008) 510–516.
[22] O. Ojurongbe, J.S. Antony, H. van Tong, C.G. Meyer, A.A. Akindele, O.R. Sina-Agbaje, et al., Low MBL-associated serine protease 2 (MASP-2) levels correlate with urogenital schistosomiasis in Nigerian children, Trop. Med. Int. Health (2015) http://dx.doi.org/10.1111/tmi.12551.
[23] I. Evans-Osses, A. Mojoli, M.H. Beltrame, D.E. da Costa, W.D. DaRocha, T.P. Velavan, et al., Differential ability to resist to complement lysis and invade host cells mediated by MBL in R4 and 860 strains of *Trypanosoma cruzi*, FEBS Lett. 588 (2014) 956–961.
[24] A.N. Jha, P. Sundaravadivel, V.K. Singh, S.S. Pati, P.K. Patra, P.G. Kremsner, et al., MBL2 variations and malaria susceptibility in Indian populations, Infect. Immun. 82 (2014) 52–61.
[25] P.R. Luz, A.B. Boldt, C. Grisbach, J.F. Kun, T.P. Velavan, I.J. Messias-Reason, Association of L-ficolin levels and FCN2 genotypes with chronic Chagas disease, PLoS One 8 (2013), e60237.
[26] A. Mishra, J.S. Antony, P. Sundaravadivel, H.V. Tong, C.G. Meyer, R.D. Jalli, et al., Association of ficolin-2 serum levels and FCN2 genetic variants with Indian visceral leishmaniasis, PLoS One 10 (2015), e0125940.
[27] D.P. Alonso, A.F. Ferreira, P.E. Ribolla, I.K. de Miranda Santos, do Socorro Pires e Cruz, Aecio de CF, et al., Genotypes of the mannan-binding lectin gene and susceptibility to visceral leishmaniasis and clinical complications, J. Infect. Dis. 195 (2007) 1212–1217.
[28] S.P. Singh, D.C. Reddy, M. Rai, S. Sundar, Serious underreporting of visceral leishmaniasis through passive case reporting in Bihar, India, Trop. Med. Int. Health 11 (2006) 899–905.
[29] S.P. Singh, D.C. Reddy, M. Rai, S. Sundar, Serious underreporting of visceral leishmaniasis through passive case reporting in Bihar, India, Trop. Med. Int. Health 11 (2006) 899–905.
[30] A. Assaf, T.V. Hoang, I. Faik, T. Aebischer, P.G. Kremsner, J.F. Kun, et al., Genetic evidence of functional ficolin-2 haplotype as susceptibility factor in cutaneous leishmaniasis, PLoS One 7 (2012), e34113.
[31] H.S. Mohamed, M.E. Ibrahim, E.N. Miller, J.K. White, H.J. Cordell, J.M. Howson, et al., SLC11A1 (formerly NRAMP1) and susceptibility to visceral leishmaniasis in the Sudan, Eur. J. Hum. Genet. 12 (2004) 66–74.
[32] S. Mehrotra, M. Fakiola, A. Mishra, M. Sudarshan, P. Tiwary, D.S. Rani, et al., Genetic and functional evaluation of the role of DLL1 in susceptibility to visceral leishmaniasis in India, Infect. Genet. Evol. 12 (2012) 1195–1201.
[33] M. Fakiola, A. Mishra, M. Rai, S.P. Singh, R.A. O'Leary, S. Ball, et al., Classification and regression tree and spatial analyses reveal geographic heterogeneity in genome wide linkage study of Indian visceral leishmaniasis, PLoS One 5 (2010), e15807.

[34] A. Sakthianandeswaren, S.J. Foote, E. Handman, The role of host genetics in leishmaniasis, Trends Parasitol. 25 (2009) 383–391.
[35] M. Asgharzadeh, A. Mazloumi, H.S. Kafil, A. Ghazanchaei, Mannose-binding lectin gene and promoter polymorphism in visceral leishmaniasis caused by *Leishmania infantum*, Pak. J. Biol. Sci. 10 (2007) 1850–1854.
[36] E.G. Hoal-Van Helden, J. Epstein, T.C. Victor, D. Hon, L.A. Lewis, N. Beyers, et al., Mannose-binding protein B allele confers protection against tuberculous meningitis, Pediatr. Res. 45 (1999) 459–464.
[37] K.R. Mayilyan, Complement genetics, deficiencies, and disease associations, Protein Cell 3 (2012) 487–496.
[38] M. Jacquet, M. Lacroix, S. Ancelet, E. Gout, C. Gaboriaud, N.M. Thielens, et al., Deciphering complement receptor type 1 interactions with recognition proteins of the lectin complement pathway, J. Immunol. 190 (2013) 3721–3731.
[39] D.F. Zhang, X.Q. Huang, D. Wang, Y.Y. Li, Y.G. Yao, Genetic variants of complement genes ficolin-2, mannose-binding lectin and complement factor H are associated with leprosy in Han Chinese from Southwest China, Hum. Genet. 132 (2013) 629–640.
[40] A.B. Boldt, I.J. Messias-Reason, B. Lell, S. Issifou, M.L. Pedroso, P.G. Kremsner, et al., Haplotype specific-sequencing reveals MBL2 association with asymptomatic *Plasmodium falciparum* infection, Malar. J. 8 (2009) 97.
[41] H.O. Madsen, M.L. Satz, B. Hogh, A. Svejgaard, P. Garred, Different molecular events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America, J. Immunol. 161 (1998) 3169–3175.
[42] A. Rantala, T. Lajunen, R. Juvonen, A. Bloigu, S. Silvennoinen-Kassinen, A. Peitso, et al., Mannose-binding lectin concentrations, MBL2 polymorphisms, and susceptibility to respiratory tract infections in young men, J. Infect. Dis. 198 (2008) 1247–1253.
[43] J.S. Antony, O. Ojurongbe, T.H. van, E.A. Ouf, T. Engleitner, A.A. Akindele, et al., Mannose-binding lectin and susceptibility to schistosomiasis, J. Infect. Dis. 207 (2013) 1675–1683.
[44] S. Hamdi, R. Ejghal, M. Idrissi, S. Ezzikouri, M. Hida, L. Soong, et al., A variant in the promoter of MBL2 is associated with protection against visceral leishmaniasis in Morocco, Infect. Genet. Evol. 13 (2013) 162–167.
[45] I.J. de Messias-Reason, A.B. Boldt, A.C. Moraes Braga, S.E. Von Rosen Seeling, L. Dornelles, L. Pereira-Ferrari, et al., The association between mannan-binding lectin gene polymorphism and clinical leprosy: new insight into an old paradigm, J. Infect. Dis. 196 (2007) 1379–1385.
[46] P. Garred, F. Larsen, H.O. Madsen, C. Koch, Mannose-binding lectin deficiency—revisited, Mol. Immunol. 40 (2003) 73–84.
[47] D.M. Mosser, S.K. Burke, E.E. Coutavas, J.F. Wedgwood, P.J. Edelson, *Leishmania* species: mechanisms of complement activation by five strains of promastigotes, Exp. Parasitol. 62 (1986) 394–404.
[48] M. Dominguez, I. Moreno, M. Lopez-Trascasa, A. Torano, Complement interaction with trypanosomatid promastigotes in normal human serum, J. Exp. Med. 195 (2002) 451–459.
[49] F.S. Noronha, A.C. Nunes, K.T. Souza, M.N. Melo, F.J. Ramalho-Pinto, Differential sensitivity of New World *Leishmania* spp. promastigotes to complement-mediated lysis: correlation with the expression of three parasite polypeptides, Acta Trop. 69 (1998) 17–29.