

Mannose-Binding Lectin and Susceptibility to Schistosomiasis

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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***HYP*A 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

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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, *HYP A*, *HYP D*, *LXP A*, *LYP A*, *LYP B*, *LYQ A*, and *LYQ C*) [11]. *HYP A*, *LYQ A*, and *LYP A* are associated with high expression of MBL, whereas *LXP A*, *HYP D*, *LYP B*, and *LYQ C* 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 (\pm 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 (\pm 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 (\pm 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'-GCCAGAAAGTAGAGAGGTATTTAGCAC-3'; internal primer exon 1, forward: 5'-CAGGTGTCTAGGCACAGATGAACC-3'; and exon 1, reverse: 5'-CCAA CACGTACCTGGTTCCC-3'. In brief, 10 ng of genomic DNA was amplified in a 20- μ L volume of reaction mixture containing 1 \times 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 μ L 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 $-550H$ 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) | <i>P</i> ^a | OR (95% CI) | <i>P</i> ^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 | ... | |
| <i>MBL2</i>* 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],

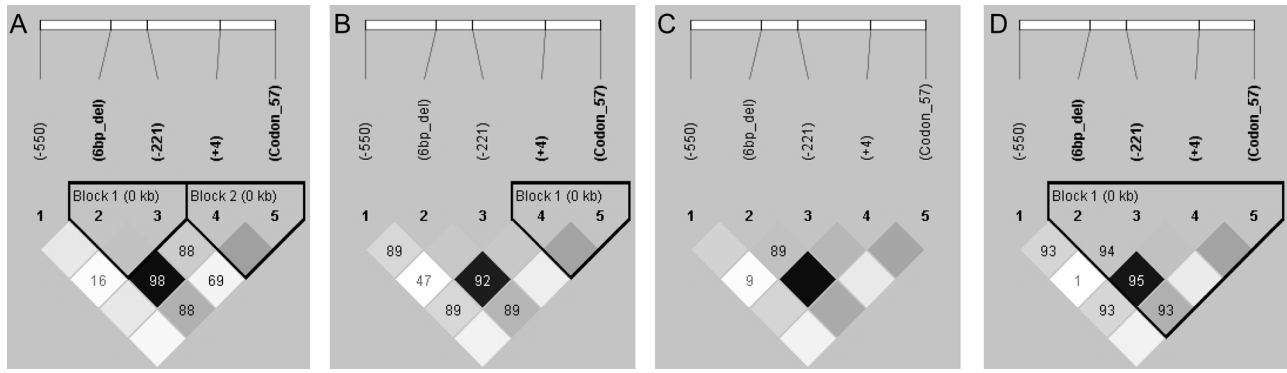


Figure 1. Linkage disequilibrium pattern of *MBL2* variants in individuals who tested positive for *Schistosoma* eggs (the SEP group [cases]; A), individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (ELISA; the SELP group [controls]; B), individuals who tested 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 2A). Also, the serum MBL levels were significantly higher in the SELP and SELN groups combined, compared with the SEP group ($P < .0001$; Figure 2A). The median MBL levels detected within groups were 27.6 ng/mL

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

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

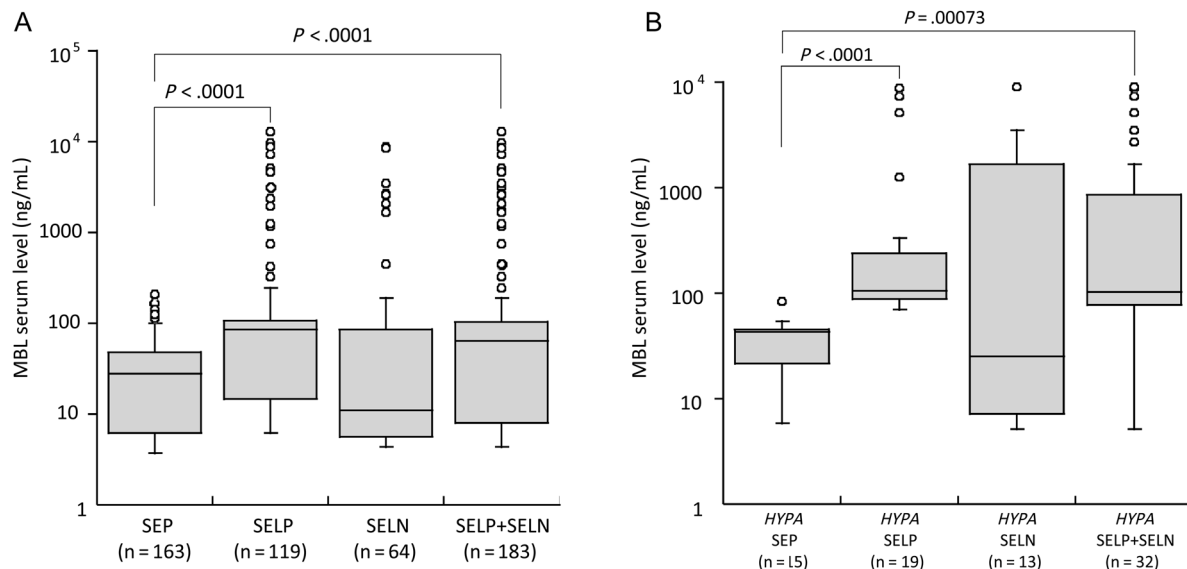


Figure 2. A, Mannose-binding lectin (MBL) serum levels in individuals who tested positive for *Schistosoma* eggs (the SEP group [cases]), individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent assay (ELISA; the SELP group [controls]), individuals who tested negative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

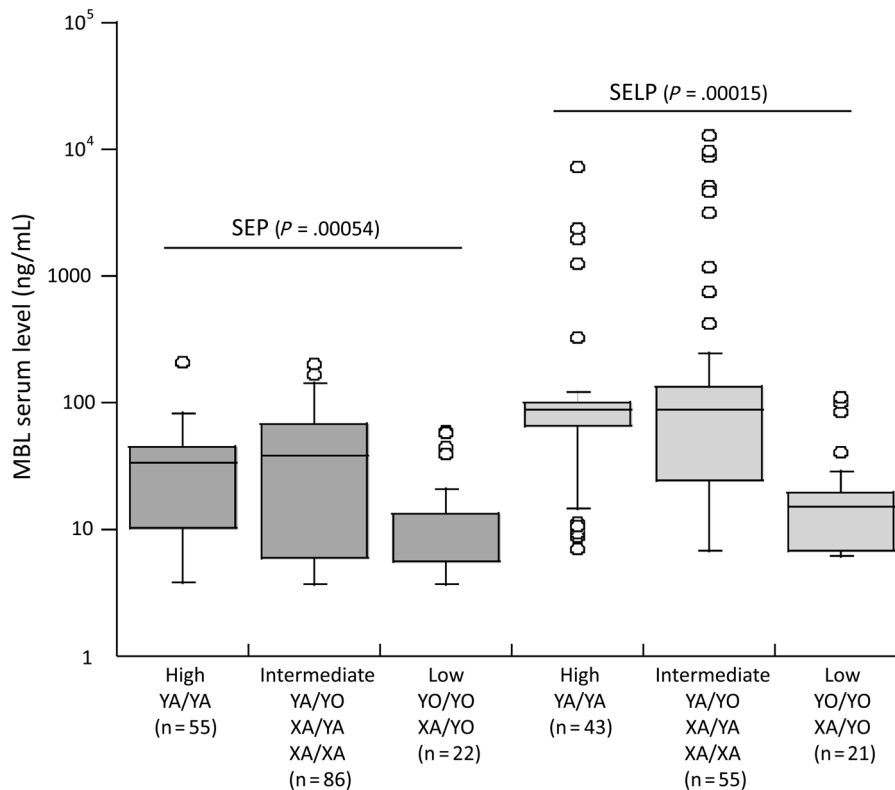


Figure 3. Mannose-binding lectin (MBL) serum levels according to combined genotypes of the -221 promoter region and exon 1 of *MBL2* in individuals who tested positive for *Schistosoma* eggs (the SEP group) and individuals who tested positive for *Schistosoma* antigens by enzyme-linked immunosorbent 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

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