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Research paper

Interleukin-4 and STAT6 promoter polymorphisms but not interleukin-10 or 13 are essential for schistosomiasis and associated disease burden among Nigerian children

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ABSTRACT

Schistosomiasis is endemic in many parts of rural Africa, with previous reports showing interleukin-13 polymorphisms as drivers of infectivity and disease severity in West Africa while IL-13/IL-4 polymorphisms contributes to patterns of reinfection in East Africa. We have shown that there is a genetic delineation in susceptibility to and severity of infectious diseases in Africa, in addition to sub-continental differences in disease pattern. Therefore, which immunoregulatory biomarkers are essential in driving *S. haematobium* infection or regulate disease burden among Nigerian school children? One hundred and thirty one age and sex-matched schistosome-infected children and 275 uninfected controls, of same ethnicity, recruited from southwestern Nigeria, were screened for variability of cytokine genes, IL-10 (*rs1800872*), IL-13 (*rs7719175*), IL-4 (*rs2243250*) and STAT6 (*rs3024974*), utilizing a polymerase chain reaction-restriction fragment length polymorphism assay. We found no difference in genotypic or allelic frequencies of IL-10 and IL-13 promoter polymorphisms alone or in association with disease. Contrariwise, we report significant differences in the frequencies of IL-4 and STAT6 variants between groups. For IL-4, the rs2243250 T/T variant was significantly different for genotypes (71.6% versus 51.2%; $p < .0004$) and alleles (82.6% versus 71.1%; $p < .001$) between disease and control groups respectively. For STAT6 (*rs3024974*), the frequencies of genotypes C/C and C/T are 75.4% and 24.6%, both showing an association with disease; none of the infected subjects had the T/T variant. Despite minor differences in disease covariates, we found no association between IL-4 and STAT6 variants with age, gender or anemia. However, mean egg count (indicative of disease burden), was regulated based on IL-4 variants, with highest burden in infected subjects with rs2243250 T/T variant (mean egg count: 207.5 eggs/10 ml of urine) versus rs2243250 C/T heterozygotes (mean egg count: 84.3 eggs/10 ml of urine) versus rs2243250 C/C (mean egg count: 127.9 eggs/10 ml of urine). Comparing rs2243250 C/T versus rs2243250 T/T ($p < .008$) or rs2243250 C/C + C/T versus rs2243250 T/T ($p < .016$) reveals an association with disease burden. We conclude that the IL-4 promoter gene is a susceptibility factor for schistosomiasis, and essential to regulate disease burden, with worse disease among carriers of the rs2243250 T/T variant. The absence of the STAT6, *rs3024974T/T* variant among infected subjects reveal the necessity of the STAT6 promoter gene in driving susceptibility to schistosomiasis in Nigeria.

1. Introduction

Schistosomiasis is prevalent in many parts of rural Africa, Latin and South America and Asia, leading to severe morbidity and negative health outcome in many endemic communities, causing disease forms ranging from intestinal to urinary or co-infections, depending on the parasite species and body part where the eggs are deposited. Severity of disease in many cases is related to patient's age, initial immune status

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and immune response to infection, with pathologies such as hydronephrosis, hematuria or liver damage. Schistosomiasis is known to drive a Th2 immune response, with high levels of circulating immunoglobulin E and eosinophilis, contributing to the Th1/Th2 balance and associated cytokines. Of significance, infection-associated cytokines are implicated in immune response, with inter-individual variability in cytokine gene polymorphisms influencing response and disease outcome. Such variabilities driven by host genetic diversity of encoding genes has been reported for many types of bacterial, parasitic, viral or autoimmune diseases, especially if such polymorphisms are located in promoter regions, modifying gene transcription and cytokine production in the process (Miteva and Stanilova, 2008; Sam et al., 2015). Previous work on schistosomiasis has established that interleukin-13 (rs7719175) gene promoter polymorphism is associated with *Schistosoma haematobium* infection levels (indicative of disease burden) and identification of a susceptibility haplotype among infected groups in Mali, West Africa (Isnard et al., 2011). Additionally, reports from Uganda, East Africa (Gatlin et al., 2009) identified polymorphisms that are associated with disease, alongside patterns of reinfection in such groups, postulating that single nucleotide polymorphisms of interferon-γ (+874), IL-13 (-1055) and IL-4 (-590 C/T) permit the development of resistant phenotypes to reinfection. Other reports have revealed the importance IL-10 promoter variants in regulating Th2 cytokines in schistosome infections (Adebayo et al., 2017), in a schistosomiasis-malaria co-infection model.

Beyond IL-10 and IL-13 variants driving disease patterns, other promoter gene polymorphisms have been implicated, to some degree, in immune response to urinary schistosomiasis, including IL-4, the signature Th2 cytokine, and signal transducer and activator of transcription 6 (STAT6) (He et al., 2008). Allelic polymorphism of IL-4 gene may affect cytokine synthesis post-infection, becoming potential driver of immunologic pathogenesis in schistosomiasis. STAT6 is a cytosolic protein whose phosphorylation is catalyzed by JAK kinases 1 and 2 (Wurster et al., 2000) and an essential ingredient for the functional responses of Th2 cells, inducing IL-4 and IL-13, in the process. Previous reports have associated STAT6 polymorphisms with the pathogenesis of autoimmune diseases (psoriasis), atopy and asthma (Tarutani, 2013; Lim et al., 2013). However, there is paucity of information on the role of STAT6 variants on schistosome pathogenesis, except reports suggesting major role in driving susceptibility and response to infectious agents (Koukouikila-Koussounda et al., 2013; Amoako-Sakyi et al., 2016), including urinary schistosomiasis (He et al., 2008). We have shown that the susceptibility to, and severity of infectious diseases, are driven by host genetic polymorphism of immunologic markers, displaying significant intra- and interethnic diversities, potentially delineated along sub-regional or sub-continental lines (Thomas et al., 2013; Thakur et al., 2014; Noble et al., 2015; Agyingi et al., 2016; Navarro et al., 2016; Ojurongbe et al., 2017).

Schistosomiasis is prevalent in rural Nigeria, with *S. haematobium* the most common causative agent, displaying varying degree of endemicity, from one part of the country to the other (*World Health Organization (WHO),* 2014). Children are the most commonly infected, including others whose daily activities entail contact with water bodies, contaminated with cercariae, the infective form of the parasite. Given the prevalence and severity of *S. haematobium* infection in rural Nigeria, are there similarities in the patterns of Th2 immunoregulatory biomarkers observed in Mali and Uganda? Alternatively, are these strictly coast-associated patterns between Nigeria (coastal West Africa) and Mali (inland West Africa), region-associated patterns between Nigeria (West Africa) and Uganda (East Africa), or linguistic-associated patterns (Afroasiatic versus Niger-Kordofanian versus Bantu), as postulated (Gomez et al., 2014)? Tangentially, are these biomarkers significant to regulate disease burden or other covariates of infection, individually or in combination? To this end, we elucidated the diversity and frequency of IL-10, IL-13, IL-4 and STAT6 cytokine gene polymorphisms between school-children infected with *S. haematobium* and uninfected control group, recruited from south-western Nigeria, as well as extrapolated potential association between schistosomiasis and covariates of infection (egg burden, age and packed cell volume (PCV) (marker of anemia).

Table 1
General characteristics of study population.

		Sh positive $n = 131$ (%)	*Sh* negative $n = 275$ (%)	p value
Gender	Male (%)	71 (54.2)	140 (50.9)	0.595
	Female (%)	60 (45.0)	135 (49.1)	
Age (years)	Range	3–20	3–20	
	Mean ± SD	10.7 ± 3.7	8.4 ± 3.6	< 0.0001
Weight	Mean ± SD	33.7 ± 12.7	25.8 ± 10.0	< 0.0001
PCV	Mean ± SD	36.9 ± 5.3	37.2 ± 5.3	< 0.0001
Parasite	Mean ± SD	178.8 ± 270.3	NA	
Count	Number with heavy infection (≥50 eggs/10 ml urine) (%)	77 (57.9)	NA	
	Number with light infection (< 50 eggs/10 ml urine) (%)	54 (40.6)	NA	

Sh: *Schistosoma haematobium*; NA: not applicable.

2. Materials and methods

2.1. Study site and subjects

This study was conducted between January and October 2015, among preschool/school age children (age 3–20 years), recruited from Ore community, Osun State, Nigeria. This community was chosen because of reports of schistosome endemicity, and a nearby water body, known to be the site of infection (Morenikeji et al., 2014). Six hundred and twenty-five school children, whose parents/guardian gave informed consent and approved of their involvement participated in this study. Individual demographic information was collected while ages were retrieved from school records (Table 1).

2.2. Urinalysis and screening for S. haematobium

Universal bottles labelled with the corresponding identification number were given to the school children who gave consent to participate in the study to produce urine specimen between 10:00 AM and 2:00 PM, and to be submitted the same day, while research team remain within the school premises. Urine samples were transported to the laboratory for microscopic examination of characteristic *S. haematobium* eggs, using the nucleopore filtration technique (Adebayo et al., 2017). Briefly, 10 ml of well-mixed urine was aspirated and carefully forced through a filter membrane; filter was removed and placed on a slide, covered with cover slip and examined under a light microscope. The number of eggs on the entire filter was counted and recorded as the number of eggs per 10 ml urine. From the total slides, 10% were randomly selected and re-examined by an independent microscopist for quality control. After parasitological testing, the cohort was divided into cases (131 *S. haematobium* positive) and control (494 *S. haematobium* negative). In the control group, DNA extraction and amplification was successfully in only 275 children which were included in the final analysis (Table 1).

2.3. PCR analysis

After sterilization with an alcohol swab, finger prick blood was spotted onto filter paper (GE Healthcare Sciences) from each schistosome patient. Genomic DNA was extracted from filter paper blood spot with the Qiagen Blood Mini Kit (Qiagen Inc., Valencia, CA), with some

modifications to the manufacturer's instruction (Ojurongbe et al., 2017). Final elution volume was 100 µl and DNA samples were stored at −20 °C until further analysis.

Genomic DNA samples were amplified by polymerase chain reaction (PCR), for each of the genes under study. To screen for interleukin (IL)-10 (-592 C/A; rs1800872), IL-13 (-4702G/T; rs7719175), IL-4 (-590C/T; rs2243250) and STAT6 (rs3024974) promoter polymorphisms, previously published primers and protocols were utilized respectively (Sam et al., 2015; Isnard et al., 2011; Malutan et al., 2016; Amoako-Sakyi et al., 2016). PCR was performed using the Lucigen EconoTaq Plus Green 2× Master Mix PCR system (Lucigen Corporation, Middleton WI), in conjunction with appropriate primers (10µM concentration), and conditions optimized to 25 µl final volume. Five microliters of amplified PCR products were resolved on a 2% agarose gel and visualized under a UV transilluminator using ethidium bromide. The size of the amplicon was estimated with a GeneRuler 100 bp Plus DNA ladder (Thermo Scientific, New Jersey) and size analysis determined as previously described (Thomas et al., 2012).

2.4. Molecular genotyping

To elucidate the polymorphic variants of the individual genes under study, individual restriction fragment length polymorphism assays were utilized, with some modifications (Sam et al., 2015; Isnard et al., 2011; Malutan et al., 2016; Amoako-Sakyi et al., 2016). Briefly, 10 µl PCR product +1 µl FastDigest restriction enzyme +2 µl 10× FastDigest (FD) Green reaction buffer +17 µl water were incubated at 37 °C for 20 min (Thermo Scientific, NJ). Digested products were examined on a 3% ethidium bromide-stained agarose gel and fragment sizes analyzed with Doc-It LS Image Analysis Software (UVP Life Sciences, Upland CA). Digesting IL-13 amplified products (244 bp) with FD *RsaI* enzyme yielded no digestion (homozygous wild type; −7402G/G) or two fragments (homozygous variants, 204 & 41 bp; −7402 T/T). For the STAT6 gene, FD *PstI* restriction enzyme cleaves the C allele into two fragments of 115 and 42 bp, with the T allele remaining intact. For IL-10 promoter gene, amplified PCR products (412 bp) digested with FD *RsaI* enzyme showed no digestion (homozygous wild type variant; -592C/C), and mutant variant (snp-592A/A) producing two fragments (236 and 176 bp). For the IL-4 promoter gene, digestion of amplified products (195 bp) with FD *AvaII* enzyme yielded two fragments (175 & 20 bp; homozygous wild type -590C/C) or no digestion (195 bp; homozygous mutant – 590 T/T.

2.5. Statistical analysis

We tested the Hardy-Weinberg equilibrium for genotypic distribution of the four loci (IL-10: rs1800872; IL-13: rs7719175; IL-4: rs2243250 and STAT6; rs3024974) using the chi-square ($\chi 2$) goodness-of-fit-test, with SNP's rejected on the threshold of $p < .05$ in control individuals. Association analysis was performed for each SNP separately by Fisher's exact-test, where in different analyses the AB, and BB were given as two groups reflecting a dominant or recessive inheritance model. Differences in genotypic and allelic frequencies between *Sh*-infected and controls were assessed by odds ratio with a 95% confidence interval, as previously described (Duru et al., 2015) and statistical analyses carried out with a PERL script (Thakur et al., 2014). A binary logistic regression was used to analyze association between genetic variants and schistosomiasis, in addition to a Student *t* test used to evaluate the association between genetic variants and additional covariates (egg burden, age and PCV).

2.6. Ethics

The human protocol reported in this study was carried out in accordance with the 1964 Helsinki declaration and met institutional ethics of Ladoke Akintola University College of Medicine and the Rochester Institute of Technology. Approval for this project was obtained from the Institutional Review Committee, Osun State Ministry of Health (approval number OSHREC/PRS/569 T/131) and informed consent received from every participant or guardian before they were recruited into the study.

Table 2
Percentile frequency of IL-10 gene variants among children with and without schistosomiasis.

Gene variant		Schistosomiasis (n = 128)	Controls (n = 242)	Odds ratio (95% CI)	p value
IL-10 rs1800872	Genotype				
	CC	42 (32.8)	75 (31.0)	1.09 (0.67–1.76)	0.7258
	CA	60 (46.9)	118 (48.8)	0.93 (0.59–1.46)	0.7440
	AA	26 (20.3)	49 (20.2)	1.00 (0.56–1.76)	1
	Allele				
	C	144 (56.2)	268 (55.4)	1.04 (0.76–1.42)	0.8764
	A	112 (43.8)	216 (44.6)	0.97 (0.70–1.32)	0.8764
	Dominant				
	CC	42 (32.8)	75 (31.0)	1.08 (0.67–1.76)	0.7258
	CA + AA	86 (67.2)	167 (69.0)	0.92 (0.57–1.50)	0.7258
	Recessive				
	AA	26 (20.3)	49 (20.2)	1.00 (0.56–1.76)	1
	CC + CA	102 (79.7)	193 (79.8)	0.99 (0.57–1.77)	1

3. Results

Human schistosomiasis is endemic in sub-Saharan Africa (*World Health Organization (WHO),* 2014), with Nigeria labelled “ground zero” for the disease, including disparate prevalence of infection from different regions of the country (Ladan et al., 2011; Oniya et al., 2011; Hotez et al., 2012; Hassan et al., 2014; Adebayo et al., 2017). Despite reports of variable immune response to disease due to significant diversity in IL-13 and IL-10 promoter polymorphisms, we found no significant difference in the genotypic or allelic frequencies of IL-10, rs1800872 (Table 2) and IL-13, rs7719175 (Table 3) promoter gene polymorphisms between *S. haematobium*-infected patients and uninfected controls. Though the wild type variant was most common genotype and allele for IL-10 promoter gene and the homozygote mutant variant was the most frequent for the IL-13 promoter, there was no statistically significant difference in the percentiles between schistosome-infected and uninfected groups.

- On the other hand, we report a statistically significant difference in the genotypic and allelic frequencies of IL-4, rs2243250 (Table 4) and STAT6, rs3024974 (Table 5) promoter variants between disease and control groups. For the IL-4 gene, the rs2243250 C/C genotype was significantly less common compared to the rs2243250 T/T genotype (Table 4) (p = .0004), with similar observation in allelic frequencies (p = .001) between disease and control groups respectively. More importantly, children with the rs2243250 T/T genotype were more susceptible to schistosomiasis (OR 2.39; 95% CI = 1.44–4.04), with the odds reduced three and a half times among those carrying the rs2243250 C/C genotype (OR = 0.69; 95% CI = 0.24–1.73). There were no association between disease covariates (age or PCV) and IL-4 variants. However, mean egg

Table 3
Percentile frequency of IL-13 gene variants among children with and without schistosomiasis.

Gene variant		Schistosomiasis (n = 121)	Controls (n = 257)	Odds ratio (95% CI)	p value
IL-13	Genotype				
rs7719175	GG	4 (3.3)	8 (3.1)	1.06 (0.22–4.07)	1
	GT	44 (36.4)	93 (36.2)	1.00 (0.62–1.61)	1
	TT	73 (60.3)	156 (60.7)	0.98 (0.62–1.57)	1
	Allele				
	G	52 (21.5)	109 (21.2)	1.02 (0.69–1.15)	0.9244
	T	190 (78.5)	405 (78.8)	0.98 (0.67–1.46)	0.9244
	Dominant				
	GG	4 (3.3)	8 (3.1)	1.06 (0.22–4.07)	1
	GT + TT	117 (96.7)	249 (96.9)	0.94 (0.25–4.35)	1
	Recessive				
	TT	73 (60.3)	156 (60.7)	0.98 (0.62–1.57)	1
	GG + GT	48 (39.7)	101 (39.3)	1.02 (0.64–1.62)	1

Table 4
Percentile frequency of IL-4 gene variants among children with and without schistosomiasis.

Gene variant		Schistosomiasis (n = 109)	Controls (n = 242)	Odds ratio (95% CI)	p value
IL-4	Genotype				
rs2243250	CC	7 (6.4)	22 (9.1)	0.69 (0.24–1.73)	0.5304
	CT	24 (22.0)	96 (39.7)	0.43 (0.24–0.74)	0.0015
	TT	78 (71.6)	124 (51.2)	2.39 (1.44–4.04)	0.0004
	Allele				
	C	38 (17.4)	140 (28.9)	0.52 (0.33–0.79)	0.001
	T	180 (82.6)	344 (71.1)	1.93 (1.27–2.96)	0.001
	Dominant				
	CC	7 (6.4)	22 (9.1)	0.69 (0.24–1.73)	0.5304
	CT + TT	102 (93.6)	220 (90.9)	1.46 (0.58–4.17)	0.5304
	Recessive				
	TT	78 (71.6)	124 (51.2)	2.38 (1.44–4.04)	0.0004
	CC + CT	31 (28.4)	118 (48.8)	0.42 (0.25–0.69)	0.0004

count, indicative of disease burden, was regulated based on variants, with the highest burden in patients with rs2243250 T/T homozygote variants (mean egg count: 207.5 eggs/10 ml of urine) versus heterozygotes (mean egg count: 84.3 eggs/10 ml of urine; p = .008) versus rs2243250 C/C variants (mean egg count: 127.9 eggs/10 ml of urine; p = .02 (Fig. 1). Individuals with rs2243250 C/C or rs2243250 C/T variants significantly controlled egg burden compared to rs2243250 T/T variants.

Table 5
Percentile frequency of STAT-6 gene variants among children with and without schistosomiasis.

Gene variant		Schistosomiasis (n = 114)	Controls (n = 266)	Odds ratio (95% CI)	p value
STAT6	Genotype				
rs3024974	CC	86 (75.4)	187 (70.3)	1.29 (0.77–2.23)	0.3225
	CT	28 (24.6)	67 (25.2)	1.01 (0.56–1.65)	1
	TT	0 (0.0)	12 (4.5)	0 (0.0–0.82)	0.0213
	Allele				
	C	200 (87.7)	441 (82.9)	1.47 (0.92–2.42)	0.1027
	T	28 (12.3)	91 (17.1)	0.68 (0.41–1.09)	0.1027
	Dominant				
	CC	86 (75.4)	187 (70.3)	1.29 (0.77–2.23)	0.3225
	CT + TT	28 (24.6)	79 (29.7)	0.77 (0.45–1.30)	0.3225
	Recessive				
	TT	0 (0.0)	12 (4.5)	(0 (0.0–0.82)	0.0213
	CC + CT	114 (100.0)	254 (95.5)	NA	0.0213

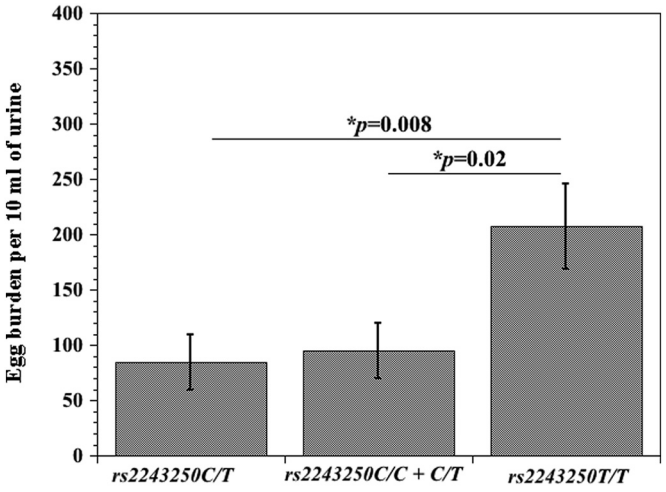

Fig. 1. Mean egg burden between IL-4 (rs2243250) variants among schistosome-infected children. rs2243250 C/T or rs2243250 C/C + C/T variants versus rs2243250 T/T) variant, showing significant difference in egg burden (p = .008 and p = .02 respectively).

For the STAT6 promoter gene, the reverse of IL-4 promoter variants was observed, despite the statistically significant difference in the genotypic and allelic frequencies between disease and control groups. The rs3024974C/C genotype was significantly more common between disease and control groups, compared to the rs3024974T/T (p = .02), which was completely absent in schistosome-infected group, potentially implying protection from infection (Table 5). The odds of infection significantly increased among individuals with the rs3024974C/C or rs3024974C/T genotypes. The frequencies of the minor allele was comparable for schistosome-infected viz-a-viz control individuals (Table 5). Despite minor differences in covariates among infected subjects, we found no association between STAT6 (rs3024974) variants with age, PCV or egg burden.

We constructed haplotype groups using polymorphisms of the 4 loci under consideration, as previously described (Duru et al., 2015),

Table 6
Estimated haplotype frequencies of the four loci between schistosome-infected patients and control groups.

Haplotype	Haplotype definition				Haplotype frequency		
	IL-10 (592C/A)	IL-4 (590C/T)	IL-13 (4702G/T)	STAT6 (C/T)	Schistosomes	Controls	Case vs control
H1	C	C	T	C	0.0706	0.1044	0.0948
H2	C	C	T	T	0.0076	0.0209	0.0163
H3	C	C	G	C	0.0199	0.0282	0.0259
H4	C	C	G	T	0.0021	0.0057	0.0044
H5	C	T	T	C	0.3155	0.2619	0.2775
H6	C	T	T	T	0.0339	0.0527	0.0476
H7	C	T	G	C	0.0892	0.0707	0.0759
H8	C	T	G	T	0.0096	0.0142	0.0130
H9	A	C	T	C	0.0581	0.0824	0.0758
H10	A	C	T	T	0.0062	0.0166	0.0130
H11	A	C	G	C	0.0164	0.0222	0.0207
H12	A	C	G	T	0.0018	0.0045	0.0035
H13	A	T	T	C	0.2599	0.2067	0.2218
H14	A	T	T	T	0.0278	0.0416	0.0380
H15	A	T	G	C	0.0735	0.0558	0.0607
H16	A	T	G	T	0.0079	0.0112	0.0104

Abbreviations: CI, confidence interval; G: guanine; A: adenine; C: cytosine; T: thymine; SNP, single nucleotide polymorphism.

producing 16 haplotype groups (Table 6). Among schistosome-infected and control groups, the most common haplotypes groups are H5 (31.5% versus 26.2%), which combines wild type STAT6 and IL-10 with mutant IL-4 and IL-13 variants, and H13 (25.9 versus 20.7%), combining wild type STAT6 with mutant IL4, IL10 and IL13 variants respectively. All other haplotypes were either very low or almost nonexistent. Haplotype H16, which combines the mutant variants for all four loci had a frequency of less than or about 1% for both infected and control groups.

4. Discussion

The benefit of molecular biology and explosion of sequencing data has led to the enhancement of our understanding on the role of host genetics in modulating susceptibility to diseases or ameliorating pathological outcomes, and the differential distributions of infectious diseases in diverse communities. Genetic variation in individuals, in many instances regulated by geography (Maisano and Fuselli, 2013; Thomas et al., 2013; Rotimi et al., 2017), ethnicity (Modiano et al., 1996; Cassiano et al., 2015; Rizzo et al., 2014), or linguistics (Gomez et al., 2014; Walakira et al., 2017), as observed globally, has major implications for human adaptability and disease prevalence. Schistosomiasis is a globally distributed neglected disease, that is highly prevalent in rural sub-Saharan Africa. We believe that diversity in human genetics play a significant role in susceptibility, immune response to infection and disease outcome. T-helper 2 (Th2) cytokine genes have been shown to regulate infectivity and disease burden in many helminthic infections, including schistosomiasis. Of importance to our study however are the reports showing IL-10 (*rs1800872*) and IL-13 (*rs7719175*) promoter genes as drivers of susceptibility and reinfection with *S. haematobium* in one part of Africa and a different outcome in others (Isnard et al., 2011; Gatlin et al., 2009). Examining the contribution of IL-10, IL-13, IL-4 and STAT6 gene polymorphisms to *S. haematobium* infection, including extrapolating disease burden is necessary to elucidate their role in susceptibility and infection outcome. We selected these genes because of their significant roles in driving Th2 immune response or T cell regulatory functions, including reports of associations between their promoter variants and disease.

We examined the promoter polymorphisms of IL-10 (-592C > A; *rs1800872*) and IL-13 (−4702G > T; *rs7719175*) genes and found no statistical difference in the genotypic or allelic frequencies between schistosome-infected and uninfected controls or any association with disease. This is contrary to a report from Brazil that found significant association between IL-10 polymorphisms and infection intensity in a schistosome-infected population (Grant et al., 2011), with an additional report showing that protection against severe infection with *Schistosoma mansoni* was driven by functional IL-13 polymorphisms (Grant et al., 2012). These reports, in contrast with our studies, reveal a possible bias due to differences between urinary and intestinal schistosomiasis. Furthermore, the fact that admixture in the Brazilian population (Thomas et al., 2013; Cassiano et al., 2015) may counteract the observation from Nigeria, despite the phenotypic similarity, is a possibility requiring further elucidation. Alternatively, this lack of association with disease or infection severity corresponds to a recent report showing that IL-10 (−1082G > A) polymorphism had no effect on schistosomiasis or co-infection with malaria (Adedoja et al., 2018). This is significant because though this study was carried out in North-central Nigeria, the location is close with an overlap in geography and ethnicity to our study, adding significance to our results. Though reports of an association between IL-13 (*rs7719175*) gene promoter and *S. haematobium* infection levels, identifying a susceptibility haplotype in inland West Africa (Kouriba et al., 2005; Isnard et al., 2011) and patterns of reinfection in East Africa exists (Gatlin et al., 2009), Nigeria is located in coastal West Africa (Niger-Congo speakers), while Mali is inland West Africa (Nilo-Saharan speakers), and Uganda in East Africa (Bantu speakers). Our report lends credence to the extensive genetic variation, across sub-Saharan Africa (Navarro et al., 2016; Walakira et al., 2017), potentially stratified along linguistic lines (Gomez et al., 2014), and strengthening the imperative to design control programs based on local epidemiological data. Our data also adds to existing literature on the role or otherwise of IL-10 and IL-13 promoter variants in conferring protection or modulating immune response to schistosomiasis.

Elucidating IL-4 (-590C > T; *rs2243250*) gene promoter polymorphism revealed a statistically significant difference in genotypic and allelic frequencies of variants between disease and control groups. The mutant variant, *rs2243250 T/T*, showed higher frequencies, significantly different between genotypes (72.3% versus 57.4%; $p = .026$) and alleles (82.7% versus 73.6%; $p = .024$), for disease and control groups respectively. Though a report from East Africa found heterozygous IL-4 -590 genotype C/T was significantly correlated with resistance to reinfection (Gatlin et al., 2009), our results show that IL-4 is a susceptibility factor driving infection with *S. haematobium* among school children in southwestern Nigeria. The increased odds of infection among children with the mutant variant demonstrates the importance of this gene. Significantly, we found mean egg count, indicative of disease burden, to be regulated based on IL-4 variants, with the highest burden in patients with mutant variants (mean egg count: 41,493 eggs/10 ml of urine) versus heterozygotes (mean egg count: 16,863 eggs/10 ml of urine) versus wild type (mean egg count: 25,571

eggs/10 ml of urine). Patients with the heterozygote (*rs2243250 C/T*) or a combination of wild type and heterozygote (*rs2243250 C/C + C/T*) variants significantly controlled egg burden ($p < .008$ and $p < .016$ respectively), compared to mutant variants (*rs2243250 T/T*), implying an association between IL-4 variants and infection intensity. Examining patient demographics and other disease covariates (age, weight, height, gender or anemia) reveal no association between schistosomiasis and IL-4 variants. We show that interleukin-4 promoter gene is a major contributor to disease susceptibility and its variants associated with disease intensity.

Our results showing an association between STAT6 gene promoter polymorphisms and schistosomiasis among school children in Nigeria correlates with those from Mali (He et al., 2008), confirming STAT6 as a susceptibility factor for infection. The fact that none of the infected individuals had the *rs3024974 T/T* variant strengthens this assertion, further confirming STAT6 as, or one of a set of genes, driving Th2 differentiation in worm infections. The comparable mean egg burden between individuals with the STAT6 wild type (C/C) or heterozygote (C/T) variant in our study is contrary to previous reports (He et al., 2008) showing a control of *S. haematobium* infection levels. The potential that this difference may be driven by host genetics or other geography-related factors should be considered in future studies. Additionally, the small sample size for our schistosome-infected group, analyzed for STAT6 association, also require further examination. The absence of any haplotypic difference between disease and control groups but for H5 and H13, which included IL-4 mutant variant, confirms the significance of interleukin-4 in regulating disease burden among Nigerian school children with schistosomiasis.

Signal Transducer and Activator of Transcription (STAT)-6 is one of seven STAT members of the JAK/STAT signaling pathway, forming diverse complexes that results in differential gene expression patterns. IL-4 and IL-13 are Th2 cytokines, serving as activators of STAT6, modulating the latter's role in specific T cell or regulatory response to infections. Therefore, over- or under-production of these cytokines may lead to dysregulated STAT6 expression, with the concomitant negative effect on immune response. This is particularly important considering the recent report of extensive and significantly delineated microbiome in urinary schistosomiasis, potentially contributing to pathological complications (Adebayo et al., 2017). The need to critically elucidate the contribution of host genetics in driving microbiome diversity, immune response and disease outcome in urinary, intestinal or co-infected schistosomiasis, and in diverse population groups cannot be overemphasized. We reiterate that IL-4 variants, showing significant diversity in our study, could ameliorate or complicate disease or disease burden based on serum STAT6 levels. A useful strategy to dissect the role of these cytokines in our disease system would be to quantify the levels of circulating IL-4, IL-10, IL-13 and STAT6 vis-à-vis STAT6 and IL-4 genetic variants, and possibly replicating this in other population groups for a definitive conclusion on the role of STAT6/IL-4 in schistosomiasis.

We conclude that the IL-4 (-590C > T; *rs2243250*) and STAT6 (rs3024974) promoter genes are susceptibility factors for schistosomiasis among schoolchildren in Nigeria. Cytokine IL-4 in particular is essential for regulating disease burden in patients infected with *S. haematobium*, with worse disease burden among carriers of the *rs2243250T/T* variant. Of significant interest would be to further these studies in other parts of Africa, among individuals singly or co-infected with *S. mansoni,* as well as conducting these studies in larger sample sizes and analyzing serum biomarkers associated with the genes of interest.

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Author contributions

BNT conceived and designed experiments and drafted the manuscript; SAA, AAA and OO recruited patients, obtained informed consent and collected samples; BNS, NTC, GL and BNT carried out molecular genotyping and analysis; YL, OO and BNT carried out statistical analysis; YL and OO contributed to the discussion and scientific content. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare we have no conflicts of interest.

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