Helminth Infections and Allergies in Ghana

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Cover image: The co-existence of traditional living and western lifestyle in Ghana. The picture was taken at the Ada estuary where the Volta river meets the Atlantic ocean.

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Dedicated to my father Philip Kofi Adjapong Amoah
who continues to inspire me everyday

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chapter 1

General Introduction

Global burden of allergic disease

Over the past few decades, there has been a sharp global increase in the prevalence of allergic disorders such as asthma, rhinoconjunctivitis, eczema and food allergy particularly among children [1]. Moreover, findings from the multi-centre International Study of Asthma and Allergy in Childhood (ISAAC) show large variations in the burden of allergic disease across continents and countries as well as between participating centres within the same countries [2]. The analysis of the global burden of allergic disease indicates a complex pattern over time. While most allergic conditions exhibit a general global rise [1], time trends point to a sharp increase in the prevalence of asthma symptoms in low to middle income countries (LMICs) while in high income countries, there appears to be a plateau or even a trend towards a decrease in symptom prevalence over time [4, 5] (Figure1).

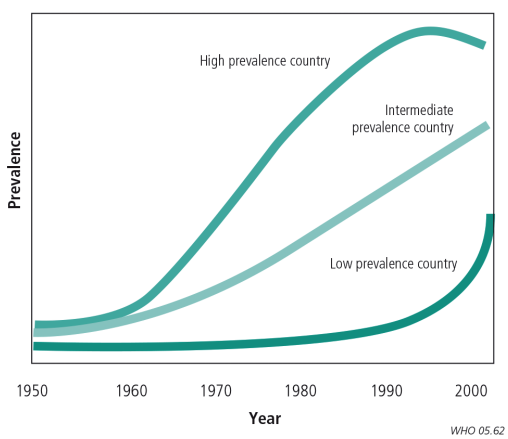

Figure 1: Global trends in the prevalence of asthma.

The lowest prevalence of asthma symptoms is seen in low to middle income countries where time trends are pointing to a sharp increase while in high prevalence countries which are also high income nations, there are indications of a plateau or decrease in prevalence over time.

Source: Bousquet J *et al.*, The public health implications of asthma. Bulletin of the World Health Organization. 2005 Jul;83(7):548-54.

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Although there is less information on the burden of food allergy from a global perspective, the point prevalence of self-reported food allergy in Europe is estimated to be about 6% with European children affected more than adults [6]. In addition, a national survey conducted in the United States estimated that among children 0 to 17 years, the prevalence of food allergy increased from 3.4% in 1997-1999 to 5.1% in 2009-2011 [7]. However, very little is known about food allergy outside of the United States, Europe and Australia. Given the growing problem of allergic disorders in relation to aeroallergy,

it would not be surprising to expect that food allergy might form the next wave of the 'allergic epidemic' [8]. Altogether, there is a rising awareness that allergic disorders need more attention in LMICs. In these countries, it is important that the extent of the burden is assessed and that there is adequate preparation to deal with the problem.

The development of allergic disease is known to be the result of complex interactions between genetic and environmental determinants [9]. In recent years, research has sought to determine the underlying factors that account for the trend towards the escalating global burden of allergic disease [1]. 'Allergic sensitization' – the production of serum-specific Immunoglobulin E (IgE) against innocuous antigens known as allergens, is a well-established factor in the pathogenesis of allergic disease [10]. Allergic sensitization can be determined by *in vitro* serological assessments as well as by *in vivo* skin tests [11] but without a positive clinical history of symptoms, it does not necessarily indicate allergic disease [12]. Interestingly, study findings show that the association between allergic sensitization and asthma symptoms in children varies greatly between populations worldwide and increases with economic development as measured by gross national income per capita [13]. In fact, for decades, economic development, urbanization and changes in lifestyle have been strongly linked to allergic disease. For example, studies from Asian economic hubs dating back to the 1970s illustrate how a higher prevalence of asthma among urban populations was associated with wealth and lifestyle changes in contrast with a lower prevalence of asthma in rural environments [14]. However, the specific factors associated with lifestyle changes and wealth which are responsible for the increase in allergies, remain unknown. In rapidly urbanizing developing countries currently, the reduction in infectious diseases especially among the affluent as well as improved hygiene and the adoption of a so-called "western lifestyle" which is also reflected in food intake, are all thought to be driving the increase in allergic disorders [15].

The hygiene hypothesis

The hygiene hypothesis could provide an explanation for the observed increase in allergic disease and the relationship with improved living standards. The formulation of this hypothesis was based on observations from a national sample of British children that showed that smaller family sizes, higher standards of living and improved hygiene led to fewer childhood infections which in turn may have resulted in greater clinical expression of hay fever [16]. In immunological terms, reduced exposure to microbes during childhood is thought to lead to inadequate maturation of the immune system's regulatory arm resulting in uninhibited inflammatory responses towards harmless antigens [17, 18].

A relationship between environmental exposure to microbes and the development of allergic disease has been shown by farming studies conducted in Europe. These studies observed that European children growing up in microbe-rich traditional farming environments are less likely to suffer from asthma and allergic sensitization compared

to their urban counterparts [19]. Farming studies have been able to highlight the fact that early life exposures to microorganisms and parasites could educate the immune system in such a way that subsequent exposures to antigens that could potentially induce allergic reactions is tolerated and no allergies develop.

Helminths as immune modulators

In LMICs, parasitic infections remain highly prevalent and particularly widespread are chronic helminth infections. Helminths are eukaryotic parasites that have evolved the ability to down-regulate their host's immune responses and thus protect against their own elimination as well as reduce severe pathology in the host [20]. Over 1 billion people living in sub-Saharan Africa, the Americas and Asia are infected with one or more helminth species [21]. In these areas, helminth infections are linked to poverty and poor sanitation [22].

Interestingly, like allergic disorders, helminth infections are associated with strong T helper 2 (Th2) responses that lead to elevated levels of IgE (Figure 2) as well as increasing numbers of basophils, eosinophils and mast cells [23, 24].

Despite the similar immunological profiles, the resultant clinical outcomes are markedly different. During an allergic reaction, the immediate response to an allergen involves a cascade with mast cell and basophil degranulation, the release of immune

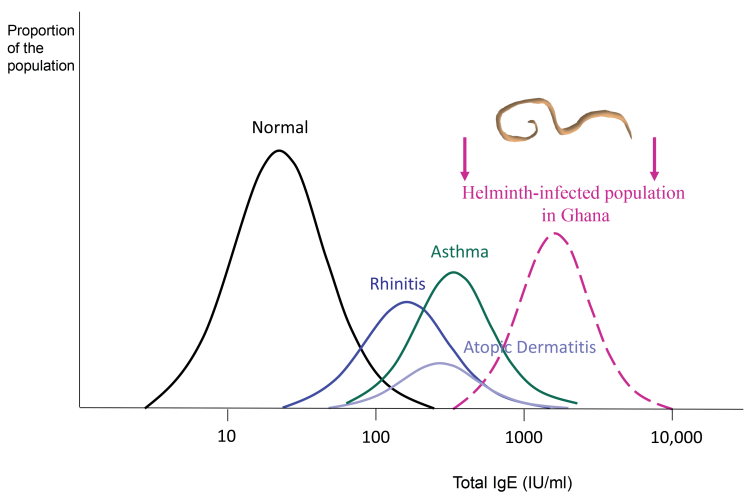

Figure 2: Total IgE in allergic disease and helminth infection.

Comparative total IgE levels in different allergic conditions and helminth infection. The figure shows a schematic representation of total IgE levels based on data from a comparative study among non-allergic and allergic Caucasian subjects in the United States (Wittig, H *et al.*, 1980 [3]). Data shown are from children aged 6 to 15 years. Figure 2 illustrates how children with rhinitis, asthma and atopic dermatitis are likely to have elevated total IgE levels compared to normal children. The figure also shows total IgE levels measured among helminth-infected children in Ghana aged 5 to 16 years.

mediators that cause an increase in vascular permeability and the contraction of smooth muscle (Figure 3A) [25]. On the other hand, these effector mechanisms are not seen in the immune response to chronic helminth infection.

Type 2 immune responses induced by helminths are characterized by the expansion of group 2 innate lymphoid cells [26] as well as Th2 cells that lead to increased production of cytokines such as interleukin 4 (IL-4), IL-5, IL-9 and IL-13 [27]. During a helminth infection, these factors are all key to the control of inflammation, enhancement of tissue repair and can result in worm expulsion [28]. Moreover, chronic helminth infections can induce an immune regulatory network in the host characterized by regulatory T cells, regulatory B cells and alternatively activated macrophages (Figure 3B) [27].

The result is an anti-inflammatory environment typified by elevated levels of IL-10 and transforming growth factor (TGF)-β as well as general T-cell hyporesponsiveness [29] which is thought to enhance survival of the worms within their immunocompetent host. Therefore, in populations chronically infected with helminths, there is an attenuation of responsiveness to so-called 'bystander antigens' that include vaccines and allergens [30].

Several epidemiological studies conducted in helminth-endemic countries have reported an inverse association between the presence of helminth infections and allergic disease [31, 32]. The picture is not very clear since some investigations have observed no effect while others have shown positive associations between helminths and allergies [31, 32].

Helminths and allergies in Ghana

Despite recent control efforts, Ghana, in West Africa, remains endemic for helminths that include soil-transmitted helminths as well as both *Schistosoma haematobium* and *S. mansoni* [33, 34]. At the same time, the process of urbanization in Ghana is leading to dramatic environmental, social and lifestyle changes. Although there is insufficient information on the national burden of allergies in Ghana, recent studies indicate that the prevalence of allergic diseases is on the increase [35-37] with factors associated with urbanization being implicated in this rise [36, 38].

This is illustrated by two surveys conducted 10 years apart in one region of Ghana that showed that the prevalence of allergy markers (exercise induced bronchospasm and allergic sensitization based on skin prick test reactivity) almost doubled over the period among schoolchildren aged 9-16 years [37]. In addition, both surveys observed that markers of allergic disease were more common among affluent urban children compared to poor urban children and compared to rural participants [35, 37]. However these investigations did not examine the role of helminth infections in observed differences in allergy outcomes.

Therefore, questions still remain on the nature of the relationship between helminth infections and allergies among children in a rapidly developing LMIC like Ghana.

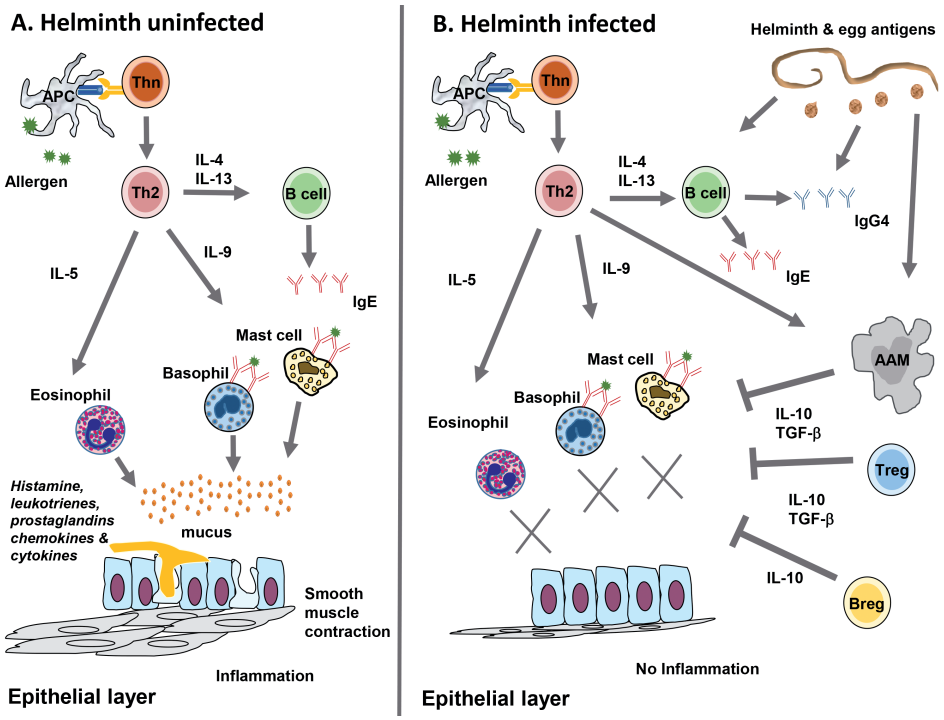

Figure 3: Allergic sensitization and airway inflammation in helminth uninfected and helminth infected individuals

A. In an uninfected individual predisposed to allergy, initial exposure to an allergen leads to the uptake and processing of the allergen by antigen presenting cells and the differentiation of CD4+ naïve T-cells into Th2 cells. Th2 cells secrete cytokines that induce immunoglobulin class switching to IgE in B cells. This process is termed allergic sensitization. Re-exposure to the sensitizing allergen triggers a cascade of events that lead to the activation of effector cells (mast cells, basophils and eosinophils) which release immune mediators such as histamine, leukotrienes, prostaglandins, chemokines and cytokines. These immune mediators induce airway inflammation in the lung epithelial layer characterized by vascular permeability, smooth muscle contraction and mucus production by goblet cells.

B. In a helminth-infected individual, the sensitization phase may occur but during chronic helminth infection, a strong regulatory network is activated involving regulatory T cells, regulatory B cells and alternatively activated macrophages. The induction of the regulatory network leads to the release of cytokines interleukin-10 (IL-10) and transforming growth factor (TGF)-β and an anti-inflammatory environment in which Th2 effector mechanisms are suppressed. Re-exposure to the sensitizing allergen does not lead to the release of immune mediators in the lung epithelial layer and therefore there is reduced airway inflammation.

Abbreviations: APC, antigen-presenting cell; Thn, CD4+ naïve T-cell; Treg, regulatory T cell ; Breg, regulatory B cell, AAM; alternatively activated macrophage;

Scope and objectives of the thesis

This thesis investigates the relationship between helminth infections and allergies among schoolchildren living in one region of Ghana.

The specific objectives are:

i. To determine urban-rural differences in allergy outcomes in Ghana
ii. To examine the association between helminth infections and allergies
iii. To characterize IgE responses associated with helminth infections and allergies
iv. To profile cellular immunological responses and their relationship with helminths and allergies in Ghana

Study design

The work described in this thesis is based on a cross-sectional study in Ghanaian children to establish the association between parasitic infections and allergy outcomes. For this investigation, urban and rural schools were approached to participate and study subjects were recruited from these schools. The urban schools were categorized as either being 'urban high socioeconomic status (SES)' which were private fee-paying schools or 'urban low SES' which were government-funded public schools. Of particular interest for the investigation, were schools in rural areas where parasitic infections were known to be prevalent and where no school-based mass deworming programmes had been implemented in recent years.

Study area and population

Out of the 10 administrative regions of Ghana, the Greater Accra Region in which the capital city is located was selected for the investigation. It is the second most populous region in the country with an estimated population of 4,010,054 [39].

According to the 2010 national household census, the proportion of Ghana's population living in urban areas is 50.9% [39] with the Greater Accra Region having the highest level of urbanization in the country [39]. At the time of the study, the region was divided into six districts and for the investigation, one urban district (Accra Metropolitan) and three rural districts (Ga West, Ga East and Dangme East) were targeted. The target age-group was children between the ages of 5 and 16 years. This age-group was sought because of its particular vulnerability to allergic disease.

Outline of the thesis

The prevalence of parasitic infections and allergy outcomes are analyzed in **Chapter 2**. In this chapter, aeroallergy as well as reported symptoms of asthma and wheeze were examined and their relationship with helminth infections was determined. In addition, the effects of body mass index as a marker of nutritional state as well as urban versus rural residence on allergy outcomes were assessed.

In **Chapter 3**, adverse reactions to food and food sensitization in Ghanaian schoolchildren were investigated for the first time. In a matched case-control analysis in a subset of participants, food sensitization based on skin prick test reactivity to food allergens and specific IgE sensitization to the same foods were examined. Reported adverse reactions to food among cases and matched controls were also assessed.

Chapter 4 focuses on peanut allergy in Ghana where the consumption of peanuts is known to be high. Adverse reactions to peanut and peanut sensitization based on serum-specific IgE as well as skin reactivity were studied. Associations between helminth infections and peanut allergy outcomes were also assessed. In a subset of the study population, the nature of peanut-specific IgE was examined through the analysis of specific IgE responses to recombinant peanut allergens as well as IgE to cross-reactive carbohydrate determinants. The chapter also explores the biological activity and cross-reactive nature of peanut-specific IgE.

The association between cellular immune responsiveness and skin prick test reactivity to house dust mite is addressed in **Chapter 5**. Immune responsiveness described in the chapter was based on cytokine responses as determined by *in vitro* whole blood culture assays.

The focus of **Chapter 6** is on urban and rural differences in the gene expression profiles of a subset of children in the study population. The role of parasitic infections in observed differences in expression profiles was also investigated. The contribution of genetic versus environmental factors in IL-10 as well as Toll-like receptor 2 and 4 expression patterns was assessed.

Chapter 7 is a review of the recent literature on helminths and allergies in childhood

In **Chapter 8**, the main study findings of the thesis are summarized and discussed along with the study limitations and future directions.

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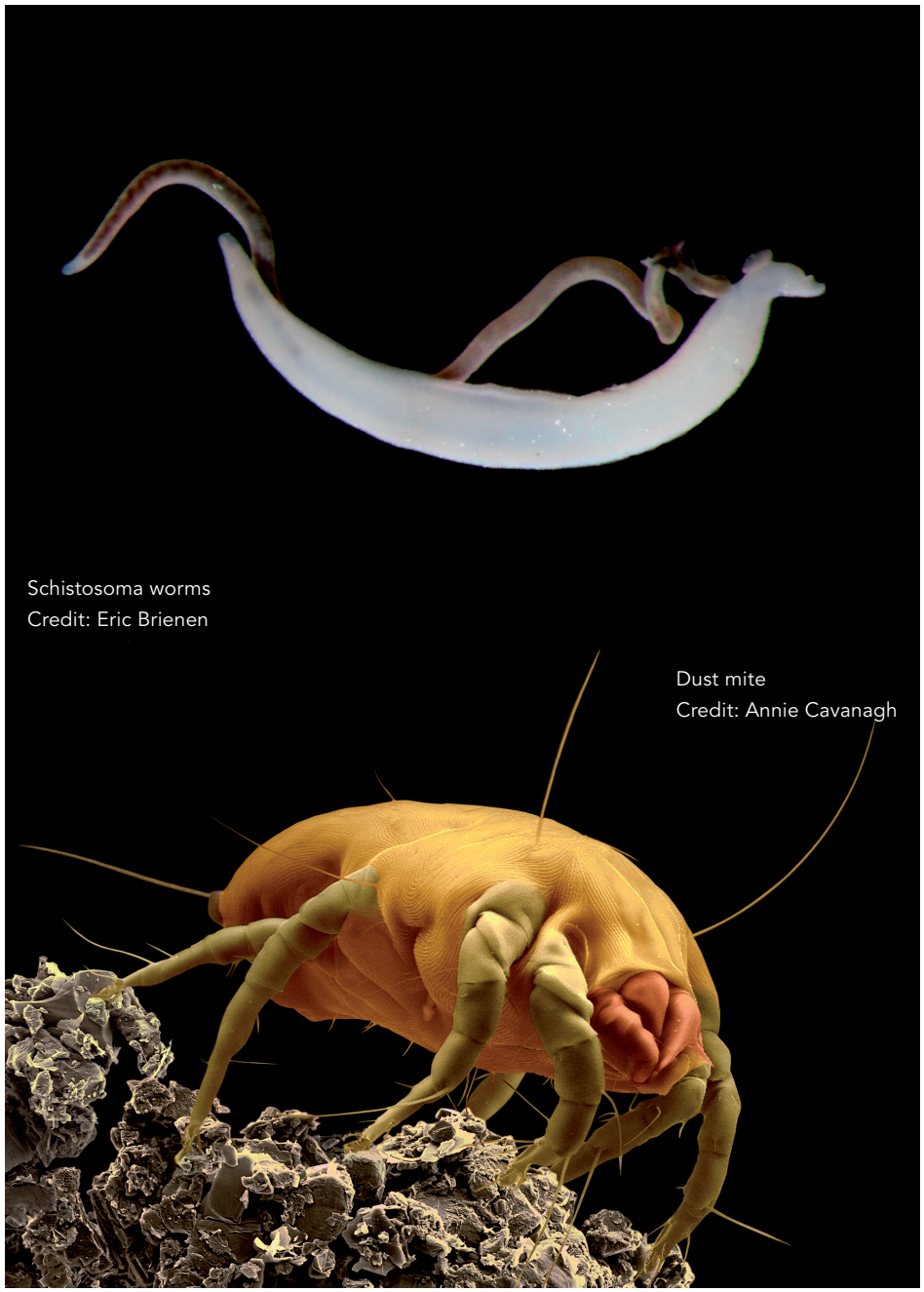

Schistosoma worms
Credit: Eric Brienen

Dust mite
Credit: Annie Cavanagh

chapter 2

Schistosome infection is negatively associated with mite atopy, but not wheeze and asthma in Ghanaian schoolchildren

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Abstract

Background: Epidemiological evidence suggests that helminth infection and rural living are inversely associated with allergic disorders.

Objective: To investigate the effect of helminth infections and urban versus rural residence on allergy in schoolchildren from Ghana.

Methods: In a cross-sectional study of 1385 children from urban high socioeconomic status (SES), urban low SES and rural schools, associations between body mass index (BMI), allergen-specific IgE (sIgE), parasitic infections and allergy outcomes were analysed. Allergy outcomes were skin prick test (SPT) reactivity, reported current wheeze and asthma.

Results: Helminth infections were found predominantly among rural subjects and the most common were hookworm (9.9%) and Schistosoma species (9.5%). Being overweight was highest among urban high SES (14.6%) compared to urban low SES (5.5%) and rural children (8.6%). The prevalence of SPT reactivity to any allergen was 18.3% and this was highest among rural children (21.4%) followed by urban high SES (20.2%) and urban low SES (10.5%) children. Overall, SPT reactivity to mite (12%) was most common. Wheeze and asthma were reported by 7.9% and 8.3% respectively. In multivariable analyses, factors associated with mite SPT were BMI (aOR 2.43, 95% CI 1.28 - 4.60, p=0.007), schistosome infection (aOR 0.15, 95% CI 0.05-0.41), and mite sIgE (aOR 7.40, 95% CI 5.62 - 9.73, $p < 0.001$) but not area. However, the association between mite IgE and SPT differed by area and was strongest among urban high SES children (aOR = 15.58, 95% CI 7.05-34.43, $p <0.001$). Compared to rural, urban low SES area was negatively associated with current wheeze (aOR 0.41, 95% CI 0.20-0.83, p=0.013). Both mite sIgE and mite SPT were significantly associated with current wheeze and asthma.

Conclusion and clinical relevance: Infection with Schistosoma appeared to protect against mite SPT reactivity. This needs to be confirmed in future studies, preferably in a longitudinal design where schistosome infections are treated and allergic reactions re-assessed.

Keywords

Africa, allergy, asthma, atopy, body mass index, cockroach, helminth, mite, rural, *Schistosoma*, urban, wheeze

Abbreviations

aOR: Adjusted odds ratio
BMI: body mass index
CI: Confidence interval
cOR: Crude odds ratio
EIB: Exercise induced bronchoconstriction
IQR: Interquartile range
OR: Odds ratio
SES: Socioeconomic status
sIgE: Specific immunoglobulin E
SPT: Skin Prick Test

Introduction

Epidemiological studies show urban-rural differences with the general increase in the prevalence of atopy in industrialized and developing countries [1-3]. Changing disease patterns associated with urbanization [4] make it necessary to identify factors involved in the growing prevalence of allergy in developing countries. Differential exposure to environmental factors, including helminth parasites, could explain some of the observed urban-rural differences [5-7]. Within urban populations, emerging trends suggest that while the incidence of allergic diseases increase with improved socioeconomic status [8, 9], poorer clinical outcomes [10] and asthma morbidity [11, 12] may be associated with poverty.

The hygiene hypothesis attributes lower incidence of allergic disorders to more frequent exchange of pathogens. Immunologically, lower exposure to T-helper (Th) 1 inducing infections allows increased Th2 responses, resulting in more allergies in affluent areas. A broader interpretation proposes that exposure to both Th1 and Th2 inducing pathogens develops regulatory responses that dampen allergies [13]. Thus, strong inducers of regulatory responses like helminths may suppress allergic reactions [14, 15].

However, conflicting results from studies in human populations show that the hygiene hypothesis alone does not explain the trends of allergic disease [16]. Recent studies, summarized in Table 1 [8, 17-24], show inconsistent results that include a lack of association between intestinal helminth infections and allergic outcomes in low helminth prevalence settings in Ethiopia [24]; an intensity-dependent inverse association between schistosome infection and atopy in Zimbabwe [17]; and a positive association between anti-*Ascaris* IgE and asthma in urban affluent subjects in Ghana [8]. High-intensity and chronic helminth infections have been suggested as being important for conferring this protective effect against allergic disease [5, 16, 17, 25-27] while lower intensity and acute infections [6, 7, 28] have been linked with exacerbated allergic outcomes. Helminth species and subject age [29] could also account for observed disparities. In addition, other confounders associated with helminth infections could mediate suppression of allergy such as diet [30], nutritional status or body mass index (BMI) [31-34], socioeconomics [8-11], and urban lifestyle [35].

The Greater Accra region (GAR) of Ghana is home to the largest city (Accra) and encompasses rural areas endemic for helminths and malaria. This region has widely varying socioeconomic status, lifestyle, urbanization and exposure to parasites. In this setting, we investigated associations between BMI, urban versus rural living, urban socioeconomic status, and different parasites, on the outcomes of atopic skin reactivity, reported current wheeze and asthma.

Table 1. Helminth prevalence and associations with allergic outcomes

Study	N	Age (years)	Helminth Type	Helminth Positive	Allergic Outcome	Association
Ghana Obeng *et al.*,¥	1385	5 to 16	*Schistosoma*	9.50%	SPT HDM	↓
			Trichuris	1.90%	SPT Cockroach	↑
			Ascaris	6.20%	SPT, Wheeze, Asthma	NS
			Hookworm	9.90%	SPT, Wheeze, Asthma	NS
Ghana Stevens *et al.*, 2012[8]	181	9 to 16	*Ascaris* IgE	52.3% $_{cases}$	Asthma	NS
				36.6% $_{controls}$	Asthma $_{Urban\ Affluent}$	↑
Zimbabwe Rujeni *et al.*, 2012[17]	672	Up to 86	*Schistosoma*	45.4%$_{high}$	*Dpt* IgE, SPT *Dpt*	↓
				8.5%$_{low}$		
Ethiopia Amberbir *et al.*, 2011[24]	878	3	Int. Helminth	9%	SPT, Eczema	NS
			Hookworm	4.90%	SPT, Eczema	NS
			Ascaris	4.30%	SPT, Eczema	NS
			Trichuris	0.10%	SPT, Eczema	NS
Ecuador Endara *et al.*, 2010[18]	3901	6 to 16	Int. Helminth	86.20%	SPT	↓
			Ascaris	57.30%	SPT	NS
			Trichuris	81.50%	SPT	↓
			Hookworm	3.90%	SPT	NS
			Onchocerca	>40%	SPT, Eczema	↑
					Wheeze , EIB	NS
Indonesia Supali *et al.*, 2010[19]	442	12 to 76	Int. Helminth	43.70%	SPT	NS
			B. malayi	46.70%	SPT Cockroach	↓
			Trichuris	14.90%	SPT HDM	NS
			Hookworm	24.20%	SPT Grass	NS
			Ascaris	22.40%	SPT Grass	NS
Vietnam Flohr *et al.*, 2010[20]	1487	6 to 17	Hookworm§	65%	SPT *Dpt*	↓
			Ascaris	7%	SPT *Dpt*	NS
South Africa Calvert & Burney, 2010[21]	749	8 to 12	*Ascaris*	34.60%	SPT, SPT *Dpt*	↓
					SPT *Btr*	NS
					EIB	↑
			Trichuris	46%	EIB	NS
Cuba Wordemann *et al.*, 2008[22]	1320	4 to 14	*Ascaris*	10%	AD	↓
			E. vermicularis†‡	22%	AD, AR	↑
			Hookworm‡	3%	AR	↑
					Asthma, SPT	NS
Brazil Rodrigues *et al.*, 2008[23]	1055	Up to 11	*Ascaris*	19.60%	SPT	↓
			Trichuris†‡	12%	SPT	↓
			Hookworm	0.60%	SPT	NS

¥ Current Study; ↓ Negative; ↑ Positive; NS Not significant;
† Current Infection; ‡ Past Infection; § IL 10 – Interleukin 10, Int = Intestinal;
AD – Atopic Dermatitis; AR – Allergic Rhinitis;
HDM – House Dust Mite; Dpt - *Dermatophagoides pteronysinnus*; Btr - *Blomia tropicalis*

Methods

Study population

The study was conducted in the Greater Accra Region of Ghana (population >4,000,000 [36]), in rural communities and the national capital Accra (Figure 1). Participating rural (R) communities endemic for intestinal helminths, *Schistosoma haematobium* and malaria [37] were; Pantang (PA) - Ga East district; Mayera (MA) and Ayikai Doblo (AD) - Ga West district; Anyamam (AN), Goi (GP), Toflokpo (TP), Agbedrafor (AB) and Koluedor (KD) - Dangme East district. Populations (and rural proportions) for Ga East, Ga West and Dangme East districts were 480,000 (18%): >300,000 (19%) and 90,000 (82%) [38] respectively. Urban schools, in the Accra Metropolis (all urban: population >1,800,000), were categorised as urban high (UH) and urban low (UL) to reflect average socioeconomic status (SES) of children attending fee-paying private and government-funded public schools respectively. Jamestown (JT), Immanuel Presbyterian (IP), and Nii Okine (NB) were UL schools, whilst Greenhill (GR) and University Primary (UP) were categorised as UH.

Subject recruitment and ethical approval

Between 2003 and 2006, 4612 schoolchildren were invited to participate in the study; 5 to 16 year old subjects were eventually recruited from thirteen schools in the communities described above. District education offices and school authorities granted permission for research in their districts and schools respectively. Initially conducted in four schools, the study was expanded to include nine additional schools. Parents and guardians agreed to participation of children by signing or thumbprinting an informed consent form after a standardized oral presentation and distribution of information letters by research staff. The Institutional Review Board of the Noguchi Memorial Institute for Medical Research in Ghana granted ethical approval.

BMI measurement

Height and weight were determined by a portable stadiometer and a scale (BS-8001, capacity: 130kg) respectively. Body mass index (BMI) was defined as weight in kilograms divided by the square of height in metres. Using previously published BMI cut-off points by Cole *et al.*, (2000, 2007) [39, 40] obtained from averaging international (2 to 18 years) data, we defined underweight as BMI of <17kg/m2 and overweight as BMI > 25 kg/m2.

Parasitology

Intestinal helminth infections were determined by the Kato-Katz technique on 25 mg sieved faecal sample per subject [41]. For *S. haematobium*, the urine filtration method was employed on 10 ml urine samples collected at mid-day [42] using 12 µm pore, 25 mm diameter nucleopore filters. Each subject provided a single stool and urine sample for these analyses. In a subset of 54 subjects, 3 samples were collected to determine sensitivity and specificity of single stool samples to hookworm infection. Helminth infection was classified

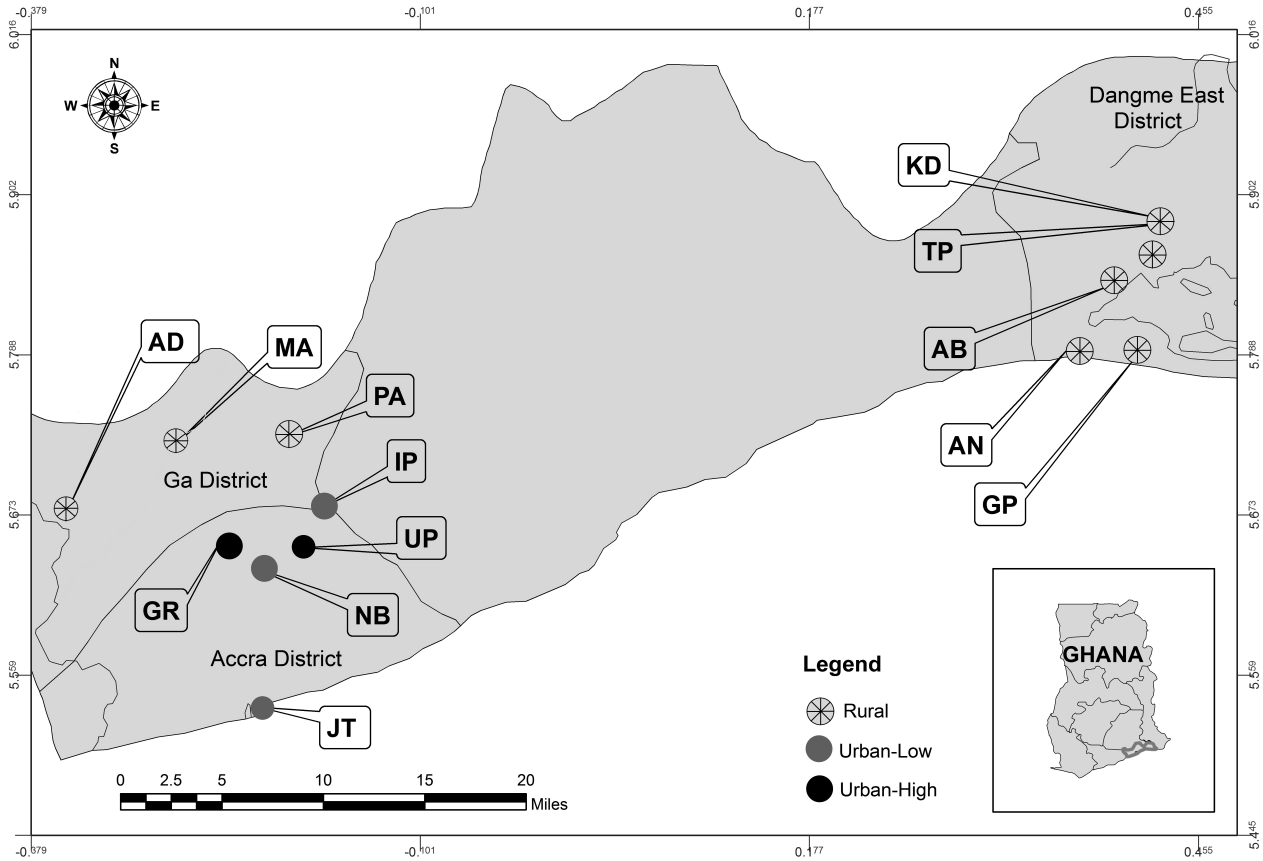

Figure 1: Map of the study area showing urban and rural schools.
Map depicts the distribution of participating schools in the Ga, Accra and Dangme East districts of the Greater Accra region of Ghana.

qualitatively by the presence of eggs and quantitatively by the number of eggs per gram of sample for intestinal helminths and per 10 ml of urine for *S. haematobium* infection. Malaria infection was determined from thick blood smears with Giemsa staining [43].

Atopy - Specific IgE sensitization and skin prick tests (SPT)

Serum allergen-specific immunoglobulin E (sIgE) antibodies to house dust mite (*Dermatophagoides pteronysinnus - Der p),* cockroach (*Blattella germanica - Bla g) and* peanut (*Arachis hypogaea - Ara h*) were measured by the Immuno-CAP™ system (Phadia AB, Uppsala, Sweden). A serum-specific IgE value ≥0.35kU/L was taken as the sensitization cut-off.

Skin tests were performed on the volar part of the left arm using 1mm standardised lancets. Dust mite species, *Dermatophagoides pteronysinnus* (Dpt) and *D. farinae* (Dfe) (HAL Allergy BV, the Netherlands) and peanut (ALK-Abelló, Denmark) were used in the first four schools. In the remaining schools, mixed mite, peanut and newly available cockroach (*Blatella germanica*) allergens (ALK-Abelló) constituted the testing panel. For analysis, a positive response to either Dpt or Dfe in the first four schools was considered as a positive SPT response to mixed mite. Diluent and histamine chloride were used as negative and positive controls, respectively. A skin reaction was assessed after 15 minutes and was considered positive when the average of the longest wheal diameter (D1) and its perpendicular length (D2) was 3 mm for the tested allergen and histamine, while that to the negative control was < 3mm. Any SPT was defined as a positive skin reaction to any of the allergens tested.

Questionnaire assessment of wheeze and asthma

A detailed questionnaire (see thesis appendix) on demographic factors, lifestyle, and socioeconomic factors, as well as on symptoms of asthma and wheeze adapted from the ISAAC Phase II questionnaire [44] was administered to parents or guardians of study participants. To minimize interviewers bias, interviewers were trained to administer questionnaires uniformly though several training sessions and by use of test questionnaires. To assess current wheeze and asthma, parents were asked the following questions:

- "Has this child had wheezing or whistling in the chest in the past 12 months?
- "Has your child ever had asthma?" or "Has a doctor ever diagnosed your child as having asthma?"

Statistical analyses

Statistical analyses included only subjects with complete data for all parasites, BMI, mite SPT and mite sIgE. Preliminary analyses involved testing prevalence differences between UH, UL and R school categories by Pearson's χ^2 at 2 degrees of freedom (4 degrees of freedom for BMI). Mean egg counts in helminth positive subjects were compared by non-parametric Kruskal-Wallis tests between rural and urban subgroups and schools.

To determine if the different manufacturer sources of mite SPT allergens gave similar results, associations between mite IgE and mite SPT were tested for heterogeneity using the inverse variance method in subjects tested with HAL and ALK allergens. Heterogeneity was also tested between urban to rural school categories for the association between IgE and SPT of like allergen.

Associations between area, BMI, parasitic infection and the outcomes of SPT, wheeze and asthma were investigated using multivariable random effects regression models to account for clustering within schools. Models were adjusted for age, sex, and log-transformed sIgE levels as *a priori* confounders. Similar associations were explored for outcomes of reported wheeze and asthma in all subjects. For measures of effect, crude and adjusted ORs and 95%CIs were generated. All statistical tests were considered significant at $p < 0.05$.

To explore the effect of multiple testing, the alpha level of 5% was divided by the total number of tests (Bonferroni correction): variables in each multivariable model and the number of outcomes. This resulted in a corrected alpha level of 5% / (7 x 4) = 0.18% for a model with 7 variables and 4 outcomes.

Statistical analyses were performed using SPSS 16.0 (SPSS Inc.), STATA version 9.2 (StataCorp, Texas, USA) and R version 2.15 (The R Foundation for Statistical Computing) software packages.

Results

Study population

A total of 2331 participants were recruited from 8 rural (n=1347), 3 urban low (n=564) and 2 urban high (n=420) schools with response rates of 68.8%, 44.8%, and 30.1% respectively. Faecal samples were provided by 86.0% (n=2013), urine by 93.2% (n=2182), and blood by 83.8% (n=1961) of the participants for analyses. Skin prick tests were performed in 2018 (86.2%) subjects for mite, 1416 (60.5%) for cockroach and 1907 (81.5%) for peanut. For complete data analyses, 1385 subjects (30% of targeted population and 59% of eligible) with data on parasites, BMI, mite SPT and mite IgE were included in analyses for this study (Figure S1).

Characteristics differed significantly between these subjects when compared to participants excluded due to missing data (n=946). Subjects included in analyses had fewer males (47.9% versus 52.6%, $p < 0.05$) and fewer *Schistosoma* positive subjects (9.5% versus 16.5%, $p < 0.001$) but more urban low (26.4% versus 20.9%, $p < 0.01$), mite SPT positive (12% versus 9%, $p < 0.05$) as well as mite IgE positive (28% versus 18.6%, $p < 0.001$) subjects.

Subject characteristics

Demographics, BMI and parasite infections:

The distributions of basic demographic, BMI, infection characteristics and outcome measures in the complete dataset are summarized in Table 2 by urban SES and rural

categories. Subjects from UL schools were significantly older but gender was evenly distributed by category. The prevalence of overweight was highest in UH schools (14.6%) compared to 5.5% and 8.6% in UL and R schools respectively. Intestinal helminths were detected in 23.1% of subjects with 92.2% of these being from the rural area. Hookworm (9.9%) was most common, compared to *Ascaris lumbricoides* (6.2%) and *Trichuris trichiura* (1.9%). Median egg counts [IQR] were *Ascaris* 62 [18-230] eggs per gram (epg); hookworm 12 [3-187] epg and *Trichuris* 7 [3-47] epg. Schistosome infection was detected in 9.5% of subjects (Table 2), the majority of which (85.5 %) were in rural schools. The intensity of infection was low, median [IQR], 21 [4–62] eggs/10 ml urine. Malaria was detected in 24.9% of all subjects (UH 3.8%, UL 6.6% and R 40%).

IgE and SPT to mite, cockroach and peanut:

Subjects with sIgE ≥0.35 kU/L ranged from 6.8% to 55.6% for mite, 4.2% to 58.3% for cockroach and 4.1% to 61.2% for peanut in individual schools. Some individual rural schools (TP) with the highest proportions of parasite infections also reported the highest proportions of atopy (Figures S2 and S3). Overall, positivity for sIgE was highest in the rural areas. Particularly for peanut, many subjects with a positive sIgE response did not have a corresponding positive peanut SPT response (Table 2). A positive SPT to any allergen was seen in 266 subjects (18.3%). Specifically, 12% reacted to mite, 10.1% to cockroach and 1.6% to peanut. There was considerable variation in SPT to mite and cockroach between schools (Figure S3A) with the lowest prevalence in the UL category (Table 2). Any SPT reactivity was comparable between UH and R school categories even though mite was predominant in UH and cockroach in rural schools (Table 2). Mite allergens for SPT from the two manufacturers HAL and ALK were similar: estimates of association between mite IgE and mite SPT were OR = 7.43, 95% CI (4.39 - 12.57) for HAL and OR = 7.37 95%CI (5.65 - 9.62) for ALK (test of heterogeneity p-value= 0.873).

Reported current wheeze and asthma:

Similar to mite and cockroach specific IgE and SPT, current wheeze was least reported in UL schools (4.0%) compared to UH (8.6%) and rural counterparts (9.2%), $p < 0.05$. There were no differences across SES and area categories in reports of asthma (Table 2).

Factors associated with mite and cockroach SPT

There was no evidence of an independent area or SES level association with mite SPT. Being overweight (adjusted OR (aOR) = 2.43, 95% CI 1.28 - 4.60, p=0.007) and schistosome infected (aOR = 0.15, 95% CI 0.05 - 0.41, $p < 0.001$) were both independently associated with mite SPT, but not significant for cockroach SPT. No intestinal helminth infection was associated with mite SPT. Increasing levels of mite specific IgE was strongly associated with mite SPT (aOR = 7.40, 95% CI 5.62 - 9.73, $p < 0.001$) (Table 3). The strongest association between mite IgE and mite SPT was in UH children (aOR = 15.58, 95% CI 7.05 - 34.43,

Table 2. Basic characteristics of subjects by SES and urban-rural categories.

	Category n (%)				
Factor	**UH = 239**	**UL = 366**	**R = 780**	**All = 1385**	**p-value**
Age					
Median [IQR] [$]	10.6 [8.7-12.1]	11.1 [9.5-12.9]	10.2 [8.7-12]	10.5 [8.9-12.1]	***
Gender					
Males	110 (46.0)	179 (48.9)	374 (47.9)	663 (47.9)	
BMI					
Underweight	8 (3.3)	30 (8.2)	25 (3.2)	63 (4.5)	***
Normal	196 (82.0)	316 (86.3)	688 (88.2)	1200 (86.6)	
Overweight	35 (14.6)	20 (5.5)	67 (8.6)	122 (8.8)	
Helminth					
Hookworm	0 (0)	5 (1.4)	132 (16.9)	137 (9.9)	***
Ascaris spp.	0 (0)	7 (1.9)	79 (10.1)	86 (6.2)	***
Trichuris spp.	1 (0.4)	7 (1.9)	19 (2.4)	27 (1.9)	
Schistosoma spp.	3 (1.3)	16 (4.4)	112 (14.4)	131 (9.5)	***
Any Intestinal Helminth	1 (0.4)	17 (4.6)	212 (27.2)	230 (16.6)	***
Any Helminth	4 (1.7)	32 (8.7)	283 (36.3)	319 (23.1)	***
Malaria	9 (3.8)	24 (6.6)	312 (40)	345 (24.9)	***
sIgE [£]					
Mite	70 (29.3)	60 (16.4)	258 (33.1)	388 (28.0)	***
Cockroach	68 (30.0)	81 (22.2)	308 (42.1)	457 (34.5)	***
Peanut	26 (10.9)	34 (9.3)	225 (28.8)	285 (20.6)	***
SPT					
Mite	39 (16.3)	33 (9.0)	94 (12.1)	166 (12.0)	*
Cockroach	17 (9.0)	16 (5.5)	78 (12.6)	111 (10.1)	**
Peanut	5 (2.5)	6 (1.6)	10 (1.3)	21 (1.6)	
Any Allergen	40 (20.2)	31 (10.5)	135 (21.4)	206 (18.3)	***
Wheeze	16 (8.6)	11 (4.0)	66 (9.2)	93 (7.9)	*
Asthma	18 (9.7)	25 (9.2)	54 (7.6)	97 (8.3)	

Significant p-value codes: *** < 0.001 ** < 0.01 * < 0.05 for Chi square or Kruskal Wallis[$] tests;
[£] Allergen sIgE ≥ 0.35kU/L.
UH - urban high, UL - urban low, R - rural;
Any intestinal helminth – Hookworm, *Ascaris*, or *Trichuris*; Any helminth – Any Intestinal helminth or *Schistosoma*

p <0.001), followed by UL (aOR = 10.44, 95% CI 5.60-19.47, p <0.001) and then rural children (aOR = 5.43, 95% CI 3.83 - 7.69, p <0.001), test for heterogeneity p=0.007.

Trichuris was positively associated with cockroach SPT (adjusted OR = 3.73, 95% CI 1.22 – 11.41, p=0.021) as shown in Table 3. Cockroach sIgE was significantly

Table 3. Factors associated with SPT reactivity to mite and cockroach

	Mite				Cockroach			
Factor	**cOR[95% CI]**	**p-value**	**aOR[95% CI]**	**p-value**	**cOR[95% CI]**	**p-value**	**aOR[95% CI]**	**p-value**
Age	1.06 [0.98 - 1.15]	0.105	1.10 [1.00 - 1.21]	0.060	**1.11 [1.00 - 1.22]**	**0.041**	1.07 [0.96 - 1.20]	0.218
Gender - Male	**1.64 [1.18 - 2.29]**	**0.003**	1.47 [0.95 - 2.27]	0.082	1.49 [0.99 - 2.25]	0.054	**1.73 [1.07 - 2.79]**	**0.025**
BMI[§]								
Underweight	0.43 [0.13 - 1.42]	0.167	0.35 [0.08 - 1.48]	0.152	0.27 [0.04 - 2.00]	0.199	0.18 [0.02 - 1.48]	0.109
Overweight	**1.86 [1.14 - 3.03]**	**0.012**	**2.43 [1.28 - 4.60]**	**0.007**	1.34 [0.71 - 2.53]	0.365	1.69 [0.81 - 3.53]	0.165
Area, SES[§]								
Urban low	0.60 [0.30 - 1.23]	0.165	0.68 [0.27 - 1.71]	0.409	**0.34 [0.16 - 0.73]**	**0.006**	0.59 [0.29 - 1.20]	0.146
Urban high	1.57 [0.74 - 3.29]	0.247	0.55 [0.20 - 1.53]	0.252	0.79 [0.36 - 1.78]	0.575	1.21 [0.60 - 2.46]	0.591
Malaria	1.09 [0.73- 1.65]	0.666	1.10 [0.65 - 1.86]	0.728	1.29 [0.80 - 2.09]	0.301	1.21 [0.70 - 2.10]	0.500
Trichuris	1.68 [0.60 - 4.71]	0.322	2.26 [0.63 - 8.13]	0.212	**4.14 [1.55 - 11.03]**	**0.005**	**3.73 [1.22 - 11.41]**	**0.021**
Schistosoma	**0.40 [0.18 - 0.91]**	**0.028**	**0.15 [0.05 - 0.41]**	**<0.001**	0.78 [0.32 - 1.88]	0.577	0.49 [0.18 - 1.29]	0.147
Specific IgE	**6.76 [5.23 - 8.75]**	**<0.001**	**7.40 [5.62 - 9.73]**	**<0.001**	**5.72 [4.23 - 7.74]**	**<0.001**	**5.94 [4.34 - 8.12]**	**<0.001**

[§] Reference categories: normal BMI and rural area. Statistical significance (in bold) is set at $p < 0.05$.
To explore effects of multiple comparisons, p-value is set at < 0.0018.
Adjusted ORs are for mutually adjusted variables (each model has age, gender, BMI, area, individual or composite parasite variables and sIgE).
Specific IgE shows associations with SPT to like allergen.
Hookworm, *Ascaris*, any intestinal helminth and any helminth showed no associations with tested outcomes and are excluded from table for simplicity.

associated with cockroach SPT (OR 5.94, 95% CI 4.34- 8.12, p <0.001), but in contrast to mite atopy, the associations were similar for rural-urban and SES category. Malaria infection was not associated with any SPT allergen. After correcting for multiple comparison, only the effects of *S. haematobium*, and sIgE on SPT of like allergen remained significant at p <0.0018 (Table 3).

Factors associated with reported current wheeze and asthma

The UL school category was independently associated with reported current wheeze compared to rural children (adjusted OR = 0.41, 95% CI 0.20 – 0.83, p=0.013) (Table 4). The UH category was not associated with wheeze or asthma. Mite atopy was positively associated with current wheeze, (SPT, adjusted OR = 3.87, 95% CI 2.33 – 6.41, p <0.001 and IgE, OR = 2.08, 95% CI 1.67 – 2.59, p <0.001). Neither cockroach atopy nor parasite infections were associated with current wheeze.

Age (adjusted OR = 0.87, 95% CI 0.79 – 0.96, p=0.005) and malaria, (adjusted OR = 0.50, 95% CI 0.27 – 0.93, p=0.027) were negatively associated with asthma. Like current wheeze, mite (but not cockroach atopy) was associated with asthma (Table 4). For both wheeze and asthma, only mite atopy remained significantly associated with these outcomes after correction for multiple comparisons.

Discussion

In this study of Ghanaian schoolchildren, we showed that the prevalence of skin test reactivity was not significantly different between urban and rural areas. Despite the similarity in SPT prevalence, the association between mite specific IgE and SPT was strongest in wealthier urban subjects and weakest in the rural. Schistosome infection, common in rural but virtually absent in higher SES urban areas, was negatively associated with mite SPT. In contrast, infection with *Trichuris* showed a positive association with cockroach SPT. Overweight was most prevalent in higher SES urban schools and significantly associated with mite skin reactivity. Mite SPT was strongly associated with reported wheeze and asthma. However, helminth infection, area and being overweight were not associated with wheeze or asthma.

The prevalence of skin test reactivity varied in neighbouring communities, but did not differ significantly between rural and urban areas once covariates were accounted for. Addo-Yobo *et al*. [3] in contrast to our study but in line with studies from South Africa, Congo [45] and Kenya [46], showed a decreasing urban-rural trend with SPT and exercise-induced bronchospasm (EIB) in Kumasi, the second largest city in Ghana. Subjects were similarly categorised by location and socioeconomic status, but the settings are geographically and culturally different from our study. One possibility for the discrepancy could be that the urban-rural classification in general is too simple to address socio-economic and cultural differences important for atopy. Particularly in the Greater Accra setting of our study, pockets of self-driven developments, alteration in

Table 4. Factors associated with current wheeze and asthma

	Current Wheeze				Asthma			
Factor	**cOR[95% CI]**	**p-value**	**aOR[95% CI]**	**p-value**	**cOR[95% CI]**	**p-value**	**aOR[95% CI]**	**p-value**
Age	0.93 [0.85 - 1.02]	0.124	0.91 [0.82 - 1.00]	0.060	**0.89 [0.82 - 0.98]**	**0.017**	**0.87 [0.79 - 0.95]**	**0.005**
Gender	1.50 [0.98 - 2.30]	0.064	1.29 [0.82 -2.01]	0.268	**1.56 [1.02 - 2.38]**	**0.040**	1.43 [0.93 - 2.21]	0.110
BMI [§]								
Underweight	0.24 [0.03 - 1.77]	0.161	0.29 [0.04 - 2.14]	0.224	1.92 [0.82 - 4.49]	0.132	2.05 [0.85 – 4.91]	0.109
Overweight	0.70 [0.30 - 1.66]	0.422	0.61 [0.25 - 1.47]	0.269	1.40 [0.69 - 2.83]	0.350	1.34 [0.65 - 2.77]	0.429
Area, SES [§]								
Urban low	**0.41 [0.21 - 0.79]**	**0.008**	**0.41 [0.20 - 0.83]**	**0.013**	1.32 [0.64 - 2.71]	0.450	1.36 [0.62 - 2.95]	0.440
Urban high	0.93 [0.52 - 1.64]	0.791	0.84 [0.45 - 1.58]	0.587	1.36 [0.59 - 3.15]	0.470	1.17 [0.48 - 2.85]	0.732
Malaria	0.83 [0.49 - 1.42]	0.502	0.61 [0.35 - 1.06]	0.079	**0.55 [0.31 - 0.98]**	**0.044**	**0.50 [0.27 - 0.93]**	**0.027**
Trichuris	0.53 [0.07 - 4.03]	0.541	0.40 [0.05 - 3.19]	0.388	0.55 [0.07 - 4.21]	0.563	0.53 [0.07 - 4.29]	0.556
Schistosoma	1.44 [0.75 - 2.77]	0.273	1.54 [0.78 - 2.05]	0.210	1.31 [0.66 - 2.58]	0.436	1.72 [0.84 - 3.53]	0.136
Mite IgE	**2.07 [1.68 - 2.55]**	**<0.001**	**2.08 [1.67 - 2.59]**	**<0.001**	**1.55 [1.25 - 1.91]**	**<0.001**	**1.54 [1.24 - 1.91]**	**<0.001**
Mite SPT	**3.66 [2.25 - 5.97]**	**<0.001**	**3.87 [2.33 - 6.41]**	**<0.001**	**2.52 [1.49 - 4.27]**	**<0.001**	**2.72 [1.58 - 4.70]**	**<0.001**
Cockroach IgE	**1.32 [1.02 - 1.70]**	**0.032**	1.26 [0.97 - 1.64]	0.078	1.00 [0.77 - 1.30]	1.000	1.04 [0.79 - 1.36]	0.782
Cockroach SPT	1.61 [0.83 - 3.13]	0.161	1.51 [0.77 - 2.96]	0.229	1.09 [0.51 - 2.36]	0.818	1.24 [0.57 - 2.74]	0.587

[§] Reference categories: normal BMI and rural area. Statistical significance (in bold) is set at $p < 0.05$. To explore the effects of multiple comparisons, p-value is set at < 0.0018. Adjusted ORs are for mutually adjusted variables (each model has age, gender, BMI, area, individual or composite parasite variables and sIgE or SPT).
Hookworm, *Ascaris*, any intestinal helminth and any helminth showed no associations with tested outcomes and are excluded from table for simplicity.

physical and spatial organization, limited access to amenities and changing cultural and ethnic environments, make urban-rural demarcation challenging and present different opportunities for disease [4]. These complexities within one country could also account for some conflicting findings between countries at similar levels of economic growth.

Consistent with findings from animal [14, 47] and epidemiological studies [27, 48], we showed schistosome infection was negatively associated with mite SPT. This has also been shown in Zimbabwe [17] particularly with higher intensity infections. Though we observed a tendency for a negative association between schistosome infection and cockroach SPT, this was not statistically significant. A general suppressory effect of schistosomiasis would be expected on all forms of atopy. Therefore, a lack of association between schistosome infection and cockroach SPT is likely due to reduced statistical power: though a confounder effect is possible.

Similar to some previous studies [18-20, 49], no significant association was observed in our population between hookworm or *Ascaris* and any allergic outcome - possibly due to relatively low infection intensities in our communities. However, higher burdens of *Ascaris*, *Trichuris* [23], and hookworm [20, 49] have been reported to be associated with less atopy. Endara *et al.*, (2010) [18] reported a negative association between *Trichuris* and any atopy for both light and heavy intensity infections, with a stronger association in the latter. Conversely, we found a positive association between *Trichuris* and cockroach SPT similar to observations made in Indonesia [19] and among rural Ethiopian subjects [5]. However, within the context of much lower *Trichuris* prevalence and intensity, our findings were less certain - given the wide confidence intervals.

Inconsistent associations between different helminth effects and skin test reactivity could result from unique characteristics of each parasite, intensity and timing of exposure. Low intensity infection with *Trichuris*, an exclusively intestinal helminth, may result in a stronger Th2 response in the face of a weak regulatory response compared to *Schistosoma*, a systemic infection with a strong regulatory characteristic [29]. Acute helminth infections could worsen allergy and be associated with pulmonary inflammation, while chronic worm infections suppress cell-mediated immune responses towards unrelated antigens [14, 50]. However, Feary *et al.*, (2011) [51] showed in a meta-analyses, consistent protective effect by helminths in general or by specific species against allergen skin sensitization or elevated specific IgE.

Immunoglobulin levels were the strongest predictor of skin reactivity to same allergen even though elevated sIgE levels did not always translate into SPT reactivity or clinical outcomes. Additionally, these associations varied with allergen type: mite (but not cockroach) atopy was associated with both wheeze and asthma. Vereecken *et al.*, (2012) [52] have similarly reported associations between IgE, SPT, and asthma while others show a dissociation between atopy and clinical outcomes [18, 24, 53] - a phenomenon attributed to infections. While our data can only speak to active regulation by current infection, programming by early life exposure to helminths [23] could account for some of these observations. The absence of a significant association

between cockroach atopy and wheeze or asthma could reflect these complexities or allergen specific factors important for clinical outcome in this study. Possibly, cross-reactivity plays a more important role in IgE sensitization to cockroach than mite allergens, thus accounting for poor association of cockroach with clinical outcomes. For this population, the role of helminth-induced IgE against cross-reactive carbohydrate determinants in peanut sensitization is discussed by Amoah *et al.,* [54].

Despite the association between mite atopy and reported outcomes, the protective effect of *Schistosoma* was not observed with wheeze or asthma. This could be due to a reporting of non-atopic or infection related wheeze in this population. Though earlier findings in Brazil [55], Cuba [18] and Ecuador [52] have also reported no association between helminths and allergic disease, a meta-analysis [26] showed *Ascaris lumbricoides* infection was associated with increased asthma risk, while hookworm was negatively associated with asthma. Possibly, the infection intensity in this population did not lead to observable changes at the clinical level of allergy. Interestingly, the negative association between malaria and asthma observed could result from immunosuppression and elevated IL-10 with malaria infection [56, 57].

Some schools with high helminth prevalence also had the highest prevalence of skin reactivity - an indication that individual, ethnic and lifestyle factors are involved in predisposing to atopy. We found being overweight was significantly associated with mite SPT, similar to urban South Africa [58]. Multiple studies have shown the importance of excess body weight in allergic disease [59-62]. In Ghana, higher BMI has been reported to be associated with urban affluent children in general, and with exercise-induced bronchospasm in urban poor, suburban and rural children [8]. Additionally, allergen exposure could account for high atopy prevalence to both mite and cockroach in rural communities. Cockroach atopy was most prevalent among the rural possibly due to greater exposure as a result of poorer hygiene. The indoor environment – humidity, ventilation and furnishing – is important for cockroach and mite allergen exposure and could vary broadly between individual homes. In addition, the high prevalence of helminth infection in these communities may have led to more frequent self-administered anti-helminthic treatment. Therefore, detected infections may have been recent or not chronic helminth infection postulated to induce down-modulation of allergy [63].

Lower SES urban subjects had the lowest proportions of sIgE sensitization, any skin reactivity and wheeze and remained negatively associated with wheeze after multivariable analyses. Addo-Yobo *et al.,* [3] showed atopy and EIB prevalence in urban areas differed according to SES. In Chile [64, 65], asthma symptoms were more common in subjects with lower socioeconomic status yet overcrowding was associated with less wheeze, atopy and bronchial hyperresponsiveness. Calvert and Burney [21] reported a relationship between possessing consumer items and EIB in South Africa. Additionally, the UL category in our study had the highest proportion of underweight and lowest proportion of overweight subjects. Sub-optimal nutrition in the urban poor, a less sedentary lifestyle, coupled with parasitic infections (including possible heavy

intensity infections in early life) could result in reduced immune sensitivity, whilst likely overcrowding would be associated with less hygiene and low rates of allergic outcomes.

A drawback of this study is the cross-sectional design which limits our ability to make inferences on causality and timing of exposure. A major limitation is the overall low response rate particularly in urban versus rural schools, lack of data on non-participants, and incomplete data from participants. The inability to perform non-responder analyses made it impossible to assess the potential bias introduced in the prevalence of atopy and helminths as well as the association between the two factors. While it did not address the bias in prevalence, it was reassuring to find in a stratified analysis that, the observed association between helminths and atopy was independent of urban high schools with high atopy prevalence but no helminths (data not shown). It was taken into consideration that with the number of associations tested; some of the findings might have arisen by chance. Also, some helminth infections may have been missed due to lower sensitivity from using single samples. Even though our analysis showed sensitivity was good for hookworm in single stool samples, this was not assessed for urine samples.

In conclusion, our results suggest that helminth infections, socioeconomic status and lifestyle are important factors in the prevalence of allergic diseases in Ghana. Against the backdrop of rapid urbanization in developing countries, it is crucial for research to recognize and address the potential for increasing allergic disease in these populations.

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Author Contributions

MY, DAB, LCR, RR and FH were involved in the planning and design of the study. BBO, ASA and IAL were involved in subject recruitment, field visits and laboratory analysis of parasitological samples. LCR and HU assisted with epidemiological and statistical issues, DKS worked on the database and GIS components of the study. MF provided expertise on allergens. ASA and BBO conducted the data analysis. BBO wrote the manuscript and all other authors critically reviewed and approved the final version.

Conflict of Interest

The authors have no conflict of interest to declare.

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Supplementary material

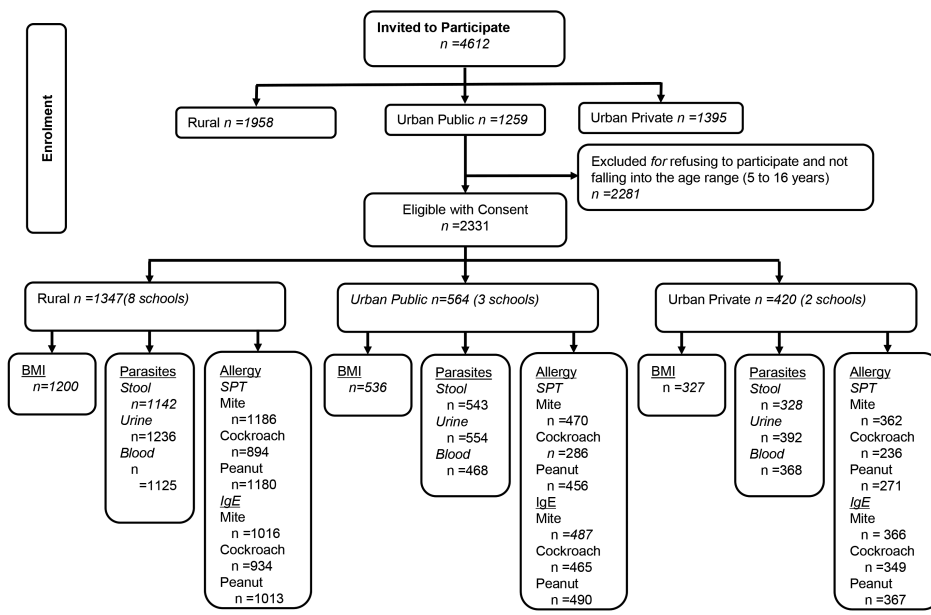

Figure S1: Study Flow Diagram

Study flow diagram detailing the number of participants targeted and the number who enrolled. Also shown is the breakdown of participants by the study parameters collected. Response rates were highest among rural schools (68.8%) and lowest in urban high SES schools (30.1%).

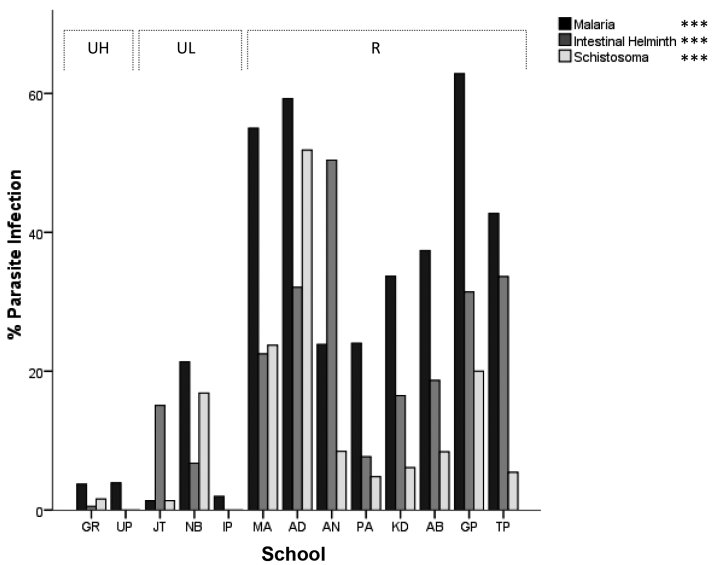

Figure S2: Parasite infection prevalence by school

Parasite infection rates in UH (Urban High), UL (Urban Low) and R (Rural) Schools. Bars represent the percentage positive rates.

P-values were calculated for χ^2 tests, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

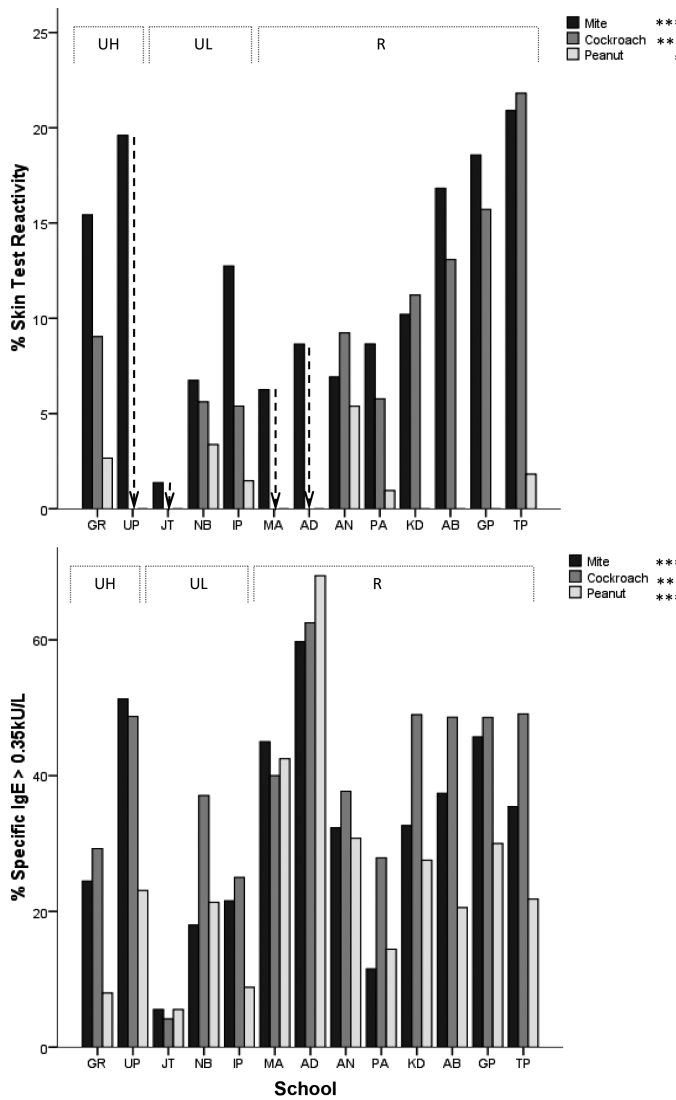

Figure S3: Atopy prevalence by school

Skin test reactivity and allergen specific IgE sensitization rates in UH (Urban-High), UL (Urban-Low) and R (Rural) Schools. Bars represent the percentage positive rates. Dashed arrows show schools for which cockroach skin test reactivity was not determined.

P-values were calculated for χ^2 tests, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

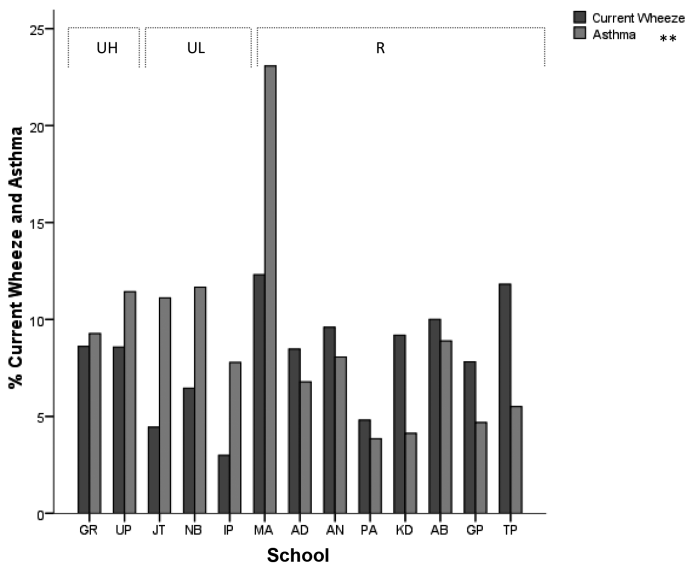

Figure S4: Reported current wheeze and asthma prevalence by school

Reported current wheeze and asthma rates in UH (Urban-High), UL (Urban-Low) and R (Rural) Schools. Bars represent the percentage positive rates.

P-values were calculated for χ^2 tests, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

chapter 3

Food allergy in Ghanaian schoolchildren: data on sensitization and reported food allergy

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Abstract

Background: Epidemiological data on food allergy are scarce in African countries. We studied the prevalence of food sensitization in Ghanaian schoolchildren.

Methods: Children (5–16 years; n = 1714) from 9 Ghanaian schools were given parental consent to participate in the study. Adverse reactions and food consumption were determined by a questionnaire and atopy by skin prick testing (SPT) to peanut and 6 fruits. Subjects with positive SPTs were considered cases (n = 43) and matched with at least 1 control (n = 84), using age, sex, and school as matching criteria. Serum samples from case-control sets were analyzed for specific IgE (sIgE) to foods that elicited a positive SPT response in cases.

Results: Overall, 11% of 1407 children reported adverse reactions to foods, and 5% of 1431 children showed a positive SPT reaction mostly directed against peanut and pineapple (both 2%). Although there was a positive association between adverse reactions and SPT responses to any food allergen in the urban children (adjusted OR = 3.6, 95% CI 1.2–10.8), most of the reported adverse reactions were not in children showing an SPT reaction to the specific food item. Specific IgE sensitization was very variable for the different foods, ranging from 0 to 100% in cases, and from 0 to 25% among controls. High IgE levels for a food item significantly increased the risk of SPT positivity to any food item in the urban, but not in the rural, schoolchildren.

Conclusions: Specific foods were identified to be allergenic in Ghana. We show a good association between SPT and sIgE in urban, but not in rural, schoolchildren. However, there was no clear association between reported adverse reactions to food and SPT or sIgE.

Key words:

Food allergy, Immunoglobulin E, schoolchildren, skin prick test, Africa

Introduction

Adverse reactions to foods are caused by several different mechanisms which could be metabolic, toxicological, or immunological in nature, or they are due to microbial contamination of the food. Food allergy is mediated by immunological mechanisms including IgE-mediated hypersensitive reactions to ingested food. The adverse reactions involved in classical IgE-mediated food allergy range from mild irritation to more severe life-threatening reactions involving the cutaneous, gastrointestinal, respiratory and cardiovascular systems. Unlike other adverse reactions to foods, food allergies are restricted to individuals who have previously been sensitized. Susceptibility to food allergy is thought to result from a combination of a genetic predisposition to allergic sensitization and exposure to food allergens [1, 2].

Food allergies are an increasing public health concern with a reported prevalence of up to 8% in young children and of 3–4% in adults in the United States, United Kingdom and Europe [3-6]. In 2008, Venter *et al*. [7] found the cumulative incidence of food hypersensitivity in 3-year-olds to be 6%; according to the authors, this indicated that the incidence of food allergy had not changed much since the study by Bock *et al*. [8] in 1987. However, earlier reports had indicated an increase in the prevalence of peanut allergy [9, 10].

Compared with other forms of allergy, such as allergy to aeroallergens, food allergy has been less extensively studied and is thus less understood. A 2004 study by Isolauri *et al*. [11] demonstrated that sensitization to dietary allergens had not followed the same consistent increase observed with sensitization to aeroallergens over several decades. Knowledge from studies on aeroallergens cannot always be applied to food allergens due to the different exposure and priming routes. Also, the reported prevalence and incidence of food allergy varies widely between locations and between assessment methods. Several studies rely on questionnaire data to assess the prevalence of food allergy [12, 13]. However, this would often include reports of other adverse reactions besides food allergy because questionnaire data are more sensitive to cultural and biased perceptions on allergy. In 2002, Woods *et al*. [14] showed that reported adverse reactions to food were an overestimation of food allergy as determined by objective methods like skin prick testing (SPT). A 2004 study by Roehr *et al*. [15] showed that the prevalence of reported perceived allergic symptoms to food was 38.4% compared to 4.2% confirmed clinically by blinded and controlled oral food challenges. This trend has also been observed in a number of other studies [7, 16].

Even when objective parameters are measured, estimation of food allergies is difficult. Individuals with elevated food allergen specific IgE (sIgE) antibodies do not always show clinical symptoms of food allergy or skin test reactivity. This has been partly explained by the cross-reactivity between airborne allergens and food allergens [17]. A recent study reported that sensitization to wheat and soy in school-aged children was mostly secondarily due to pollen sensitization [18]. In addition, while quantitative measurements of IgE antibodies to some foods like milk and eggs have been found to be useful for the evaluation of food hypersensitivity, they have shown limited value

for others like wheat and soybeans [19, 20]. The double-blind placebo-controlled food challenge (DBPCFC) is the gold standard for determining food allergy. However, it can only be performed in a proper clinical setting under expert supervision and is therefore difficult to apply in epidemiological studies, especially when experience with DBPCFC is limited or even absent. The apparent complexity of the mechanisms which underlie food allergy development further complicates the study of food allergy. In terms of studying the risk factors that govern the development of food allergy, several dietary factors in early childhood have been suggested to play a role, including the duration of exclusive breastfeeding and the age at which the infant is introduced to formula milk and complementary solid foods. However results have been inconclusive [21-23].

These limitations of the study of food allergy are particularly evident with regard to the absence of information from low-income countries, particularly in Sub-Saharan Africa. Few studies on immigrant subjects suggest that food allergy is not exclusive to natives of countries in the northern hemisphere. A particular example is a study comparing food intolerances and allergies between native Italian children and immigrant children from Africa, which showed that adverse food reactions were also a problem in immigrant African children [24]. Dias *et al*. studied food allergy among Caucasian and non-Caucasian children (including Blacks, Asians, and children of mixed race) presenting at an allergy clinic [25]. They found that the non-Caucasian children had a lower mean age at which the first food-allergic reaction occurred, had a higher average number of food allergens per child, and constituted the greater proportion of patients at the allergy clinic when compared to the general paediatric clinic. Taken together, these results highlight the need for more studies within Africa and in other parts of the world where food allergy studies are limited.

We examined the extent of reactivity to a set of food allergens in a population of schoolchildren in Ghana, West Africa. Furthermore, we studied the reported symptoms of food allergy, the serum levels of food sIgE, and the relationship with skin test reactivity. We also explored how early life factors, socioeconomic status (SES), helminth infections, and allergy to aeroallergens are related to having a positive skin reaction to food allergens.

Methods

Study Design

We conducted a matched case-control analysis on sensitization to food allergens within a cross-sectional study of allergic disorders in Ghana. The relationship between SPT using fresh foods and sIgE sensitization to those same foods, eating patterns, and reported adverse reactions to foods were examined in this subpopulation. The study was approved by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research, Ghana. Written or verbal parental consent confirmed by a signature or thumbprint were obtained for each child before we commenced with the study.

Study area and subjects

This was a cross-sectional study to assess the problem of food allergy in Ghana. It was conducted in the Greater Accra Region of Ghana between longitudes 000.35377° W and 000.42752° E and latitudes 005.72647° N and 005.53550° S. The study period was from March 2006 to March 2008. The study participants, aged between 5 and 16 years, were recruited from 3 urban and 6 rural schools which had been invited to take part in the study and had agreed. These included rural schools in Pantang (in the Ga district) and in Anyamam, Goi, Toflokpo, Agbedrafor, and Koluedor in the Dangme East district. The main income-generating activities in these rural areas are farming and fishing. The urban schools were located in the capital, Accra, in the suburbs of Madina and Achimota. Generally, activities in the urban area are more diverse and reported occupations vary from vocations like dressmaking and hairdressing, through teaching, to highly specialized jobs such as lawyers and medical doctors. Participation rates were not different between urban and rural areas (36.4% and 34.7%, respectively).

Skin prick test

Skin test reactivity to allergens was tested using the standard protocol [26]. Allergens included a commercial preparation of peanut (Alk-Abelló, Madrid, Spain), as well as fresh apple, banana, mango, orange, pawpaw, and pineapple from the local market for prick-to-prick testing [26]. These foods were selected based on availability and because they required no preparation before consumption. The allergen milk was not included since it is expensive in Ghana and only constitutes a small component of the Ghanaian diet. Soy is mostly used in infant-weaning foods which did not fall into our age range, while shellfish is also eaten in small quantities. Histamine chloride (10 mg/ml) was used as the positive control and the allergen diluent as the negative control (both controls from Alk-Abelló, Madrid, Spain). Skin prick tests were conducted on the volar side of the lower arm (avoiding the flexural and wrist areas) of the subjects using 1 mm standardized lancets. A skin reaction was considered positive when the average of the longest wheal diameter (D1) and its perpendicular length (D2) was ≥3 mm [27] for the test allergen and histamine and that to the negative control was <3 mm. Atopy was defined as a positive reaction to any of the food allergens tested.

Definition of cases and controls

Cases were defined as subjects who were SPT positive to any of the tested foods. Subjects who showed a negative response to the histamine-positive control (average wheal diameter <3 mm) were excluded. Each case was matched with at least one control of the same sex and age (± one year) from the same school. Controls were negative to all food allergens tested, with a diameter of 0 mm (complete absence of a wheal). Of the 71 atopic subjects in the cross-sectional population, 43 could be matched for age, sex, and school with at least one control based on the availability of blood samples for

IgE determination. Thirty-eight cases were successfully matched with 2 controls each, 4 cases were matched with single controls, and 1 case with 4 controls.

Questionnaire

We administered a questionnaire based on the International Study of Asthma and Allergies in Childhood (www.isaac.auckland.ac.nz) Phase II module (see thesis appendix) to the parents or guardians of the subjects to gather the demographic and socioeconomic characteristics of our study population, establish the risk factors associated with the development of various allergic orders, and investigate the reported symptoms of these allergic disorders. The questionnaire also included questions from the EuroPrevall study on the symptoms of adverse reactions to food (www.europrevall.org). The questionnaire was administered to the study participants by trained interviewers that were fluent in the local language of the participants. It included questions on early-life factors like breastfeeding duration, premature birth, birth weight, and day care attendance in the first 2 years of life, as well as daily and weekly food consumption patterns, observed adverse reactions to foods, and the amount of money spent monthly on food.

Food allergen sIgE

Food allergen sIgE antibodies against apple, banana, mango, orange, pawpaw, peanut, and pineapple were determined using ImmunoCAP™ (Phadia AB, Uppsala, Sweden) on serum samples from cases and controls. Antibody levels $\geq$0.35 kU/L were considered positive for allergic sensitization. In cases with multiple positive skin test reactions, sIgE for the different foods eliciting the responses was expressed as the total mean IgE per subject for comparison with cases with a single SPT response.

Parasitological examinations

Each subject provided one stool sample for the determination of the presence of intestinal helminth eggs using the Kato-Katz technique [28] with 25 mg of stool. The urine filtration method [29] was employed on single 10 ml urine samples from each subject to detect *Schistosoma haematobium* (urinary schistosomiasis) eggs. Urine samples were filtered using a nylon nucleopore filter (pore size 12 µm) in a swin-lok filtration device (Nucleopore, USA), and specimen slides were read by microscopy.

Statistical analysis

All data was entered into a Microsoft Access 2003 database. Analyses were performed using SPSS version 14.0 software while graphs were generated with Excel 2003 and GraphPad Prism version 5. The descriptive data are presented as frequencies, percentages, medians, and ranges. To test for significant associations between the measured variables and being a case, conditional logistic regression was performed using the Cox regression analysis

option in SPSS. To ensure that the results of the adjusted conditional logistic regression were not erroneous due to collinearity, we checked the correlation between the covariates and put them in the model only if the coefficient of correlation was less than 80% and if the measures of tolerance and the variance inflation factor did not indicate collinearity. We displayed the relative risk estimate as odds ratios (ORs) with 95% confidence intervals. Nonparametric tests were used to explore differences in sIgE levels among cases and controls because IgE data were not normally distributed.

Results

Prevalence of food allergy in the cross-sectional survey

The total number of participants with parental consent was 1714. The prevalence of reported adverse reactions assessed by the questionnaire in 1407 subjects was 11.0% in the total sample, 13.2% (of 897) in rural children, and significantly lower in urban children, i.e. 7.6% (of 510); p = 0.004. However, reported reactions to pineapple and kontomire (cocoyam leaves) opposed this general trend and were significantly higher in urban children (p <0.01). The relevant food items to which adverse reactions were reported are given in Figure 1a. The nature of the adverse reactions is summarized in Figure 1b, with diarrhoea and vomiting being the most frequent symptoms of adverse reactions in general. While symptoms involving tingling or swelling of the mouth, lips, and throat were significantly more frequent in urban subjects, all other symptoms reported were more frequent in rural subjects.

Skin reactivity to the 7 food allergens is presented in Table 1 as specific prevalence per food allergen and as overall sensitization to food. The specific foods which elicited the highest frequencies of skin reactivity were peanut (2.0%) and pineapple (2.0%). Banana and apple elicited very few positive reactions (0.4 and 0.3%, respectively) as shown in Table 1. A total of 71 subjects showed a positive skin reaction to any food allergen; 52 (73.2%) of these subjects were sensitized to single foods only. Among rural children, 4.4% had a positive SPT reaction to a food allergen compared to a prevalence of 5.9% among urban children. Furthermore, the proportion with multiple SPT positive results was 1.1% and 1.7% in rural and urban children, respectively, and the differences between rural and urban subjects were not statistically significant.

Pineapple and peanut ranked second and third (after beans) as foods to which adverse reactions were reported, and they induced the most positive SPT of all foods tested. A positive SPT response to any of the foods tested was positively associated with having a reported adverse response to any food (adjusted OR = 2.0, 95% CI 1.0–3.9) but this association was significant only in the urban subjects (adjusted OR = 3.6, 95% CI 1.2–10.8) (Table 2). For the specific food items, 4 out of 25 urban children (16.0%) with a positive SPT response to food had an adverse reaction to the specific food item, whereas this was only true for 2.9% in the rural children (1 out of 34). This difference, however, was not statistically significant.

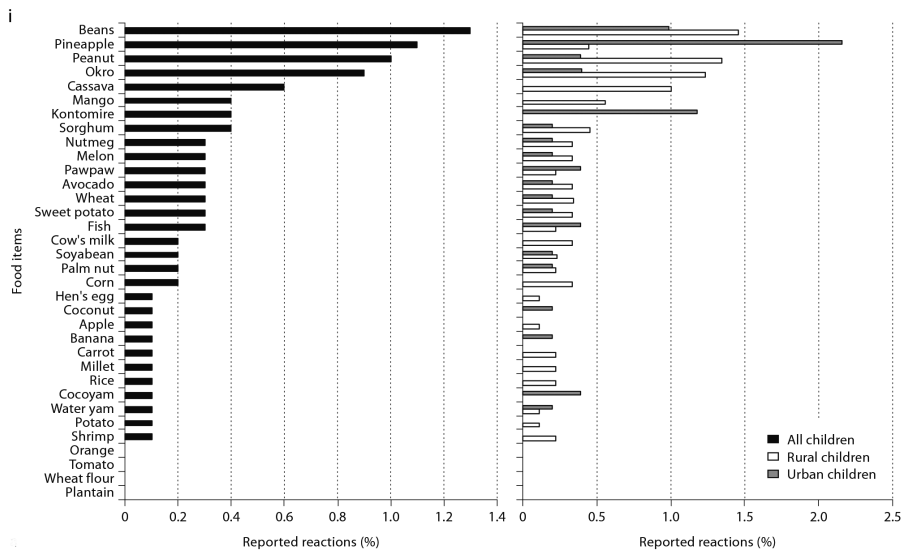

Figure 1a: Cross-sectional prevalence of reported adverse food reactions

Cross-sectional prevalence of reported adverse food reactions (n = 1407) to specific foods in all children (i) and in urban compared to rural subjects (ii). Significant urban versus rural differences were observed for pineapple and kontomire ($p < 0.01$) as well as for cassava ($p < 0.05$).

For peanut, the urban versus rural difference was borderline ($p < 0.10$).

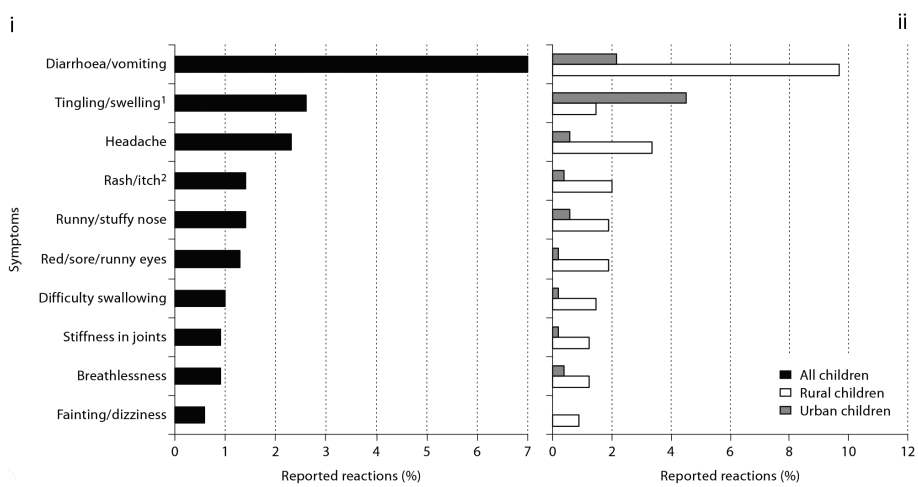

Figure 1b: Cross-sectional prevalence of symptoms of reported adverse food reactions

Cross-sectional prevalence of symptoms of reported adverse food reactions in all children (i) and in urban compared to rural subjects (ii).

[1] Tingling/swelling = swelling of the mouth, lips or throat

[2] Rash/itch = itching of the skin including nettle sting-like rash

Significant urban-rural differences were observed for diarrhoea/vomiting ($p < 0.001$), tingling/swelling, headache and red/sore/runny eyes ($p < 0.01$) as well as for rash/itch and difficulty swallowing ($p < 0.05$).

Table 1: Cross-sectional prevalence of food allergy by SPT

Food	Rural (n=906)	Urban (n=525)	Total (n=1431)
Peanut	18 (2.0)	11 (2.1)	29 (2.0)
Pineapple	16 (1.8)	12 (2.3)	28 (2.0)
Pawpaw	7 (0.8)	8 (1.5)	15 (1.0)
Orange	8 (0.9)	6 (1.1)	14 (1.0)
Mango	4 (0.4)	7 (1.3)	11 (0.8)
Banana*	1 (0.1)	5 (1.0)	6 (0.4)
Apple	2 (0.2)	2 (0.4)	4 (0.3)
Multiple foods	10 (1.1)	9 (1.7)	19 (1.3)
Any food	40 (4.4)	31 (5.9)	71 (5.0)

* P-value < 0.05 for the difference in prevalence between urban and rural subjects. Values are presented as n (%).

Table 2: Associations between SPT and reported adverse reactions to foods in a cross-sectional survey.

		Adverse reactions N (%)		
Area (N)	Any food SPT	No	Yes	Adjusted OR (95% CI)
Rural (813)	-	679 (96.0)	100 (94.3)	1
	+	28 (4.0)	6 (5.7)	1.5 (0.6 to 3.9)
Urban (409)	-	356 (94.7)	28 (84.8)	1
	+	20 (5.3)	5 (15.2)	**3.6 (1.2 to 10.8)**
Total (1222)	-	1035 (95.6)	128 (92.1)	1
	+	48 (4.4)	11 (7.9)	2.0 (1.0 to 3.9)*

Adjusted odd ratios are corrected for age, sex and school. ORs with p <0.05 are in bold and *p <0.10.

Table 3 shows the daily consumption of the foods that were tested in the SPT for the urban and rural children, separately. Consumption of orange, peanut, and banana was the most prevalent among all children (above 25%). With the exception of apple, all the other tested foods were more widely eaten among rural children than among urban children.

Measures of atopy in cases and controls

We looked for at least 1 matched control subject for each case, based on age, sex, school, and the presence of data for sIgE. This resulted in 43 cases and 84 controls. In Figure 2, we show that sIgE antibody levels were generally higher in cases than in controls. Moreover, while cases had up to 100% IgE sensitization (orange), the maximum proportion of sensitized subjects observed in controls was 25% (orange and banana). Apple was an exception, with none of the cases being sensitized. Generally, 72% of cases

Table 3: Daily consumption of the 7 foods items in the SPT

Food	Rural N (%)	Urban N (%)	Total N (%)
Orange	511 (57.3)	257 (50.7)	768 (54.9)
	892	507	1399
Pawpaw	130 (14.6)	30 (6.0)	160 (11.5)
	893	497	1390
Mango	297 (33.3)	82 (16.5)	379 (27.3)
	892	498	1390
Peanut	413 (46.3)	62 (12.2)	475 (34.0)
	892	507	1399
Apple	13 (1.5)	34 (6.7)	47 (3.4)
	894	505	1399
Banana	318 (35.6)	119 (23.5)	437 (31.2)
	894	506	1400
Pineapple	149 (16.7)	49 (9.8)	198 (14.2)
	894	500	1394

Daily consumption refers to consumption on 'most days'.
Values are presented as n (%) and total count.

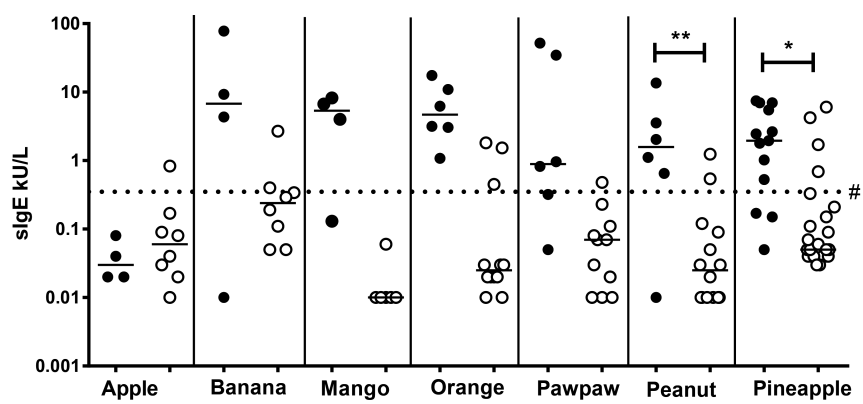

Figure 2: Median food specific IgE antibody levels in cases and controls

Median food specific IgE antibody levels in cases and controls per food item. Cases (closed symbols) had higher specific IgE levels than did matched controls (open symbols). * $p < 0.01$ and ** $p < 0.05$ by $\chi 2$ analysis. # 0.35 kU/L cut-off for IgE sensitization.

had positive sIgE responses to the specific food they reacted to in the SPT, compared to 16% of controls. This is reflected in an increased overall risk of having a positive SPT with an OR of 2.3 (95% CI 1.3–4.1) per unit increase (kU/L) in an sIgE level (OR adjusted for age, histamine wheal size, and skin reactivity to the aeroallergens cockroach and mite).

In Table 4 we show that cases who had multiple positive SPT responses (multi-atopics) had significantly higher median levels of food sIgE when compared with cases with single responses (p = 0.012, Mann-Whitney U test). All multi-atopics were sIgE-sensitized above the 0.35 kU/L cut-off to at least 1 of the relevant food items, compared to the single atopic cases with 61.3% sIgE sensitization ($p \leq 0.02$, χ^2 test). The single atopic cases without sIgE sensitization were not different in age, gender, wheal size per allergen, rural or urban location, or helminth infection compared to those with elevated sIgE levels.

Examining the possible differences between urban and rural children, we observed that controls from rural schools had a higher proportion of sIgE sensitization (24%) compared to controls from urban schools (8.5%), and they had significantly higher median levels of sIgE (Table 4). As a result, the odds of being a case predicted by the level of sIgE was much greater in the urban subjects (crude OR = 16.9, 95% CI 1.4–167.7) compared to rural subjects (crude OR = 3.4, 95% CI 1.0–11.0). Since helminth infections are associated with high IgE levels, we evaluated whether the higher levels of sIgE in the rural children were due to current helminth infections. However, the difference in sIgE levels between urban and rural children could not be explained by the presence of detectable helminth infections mainly in the rural children.

Regarding adverse food reactions, cases reported a higher prevalence (20%) compared to controls (9%), though this did not reach statistical significance in the matched analysis. The frequency of adverse reactions varied from a one-time event

Table 4: Specific IgE sensitization ≥0.35kU/L in controls and cases

					P-value	
		N	sIgE ≥0.35kU/L n (%)	Median sIgE kU/L and (range)	χ2 test[1]	#MWU/ KW Test[2]
Positive SPT	Controls (none)	84	13 (15.5)	0.05 (0 - 6.03)		
	Cases with:					
	At least one	43	31 (72.1)	2.04 (0.01 - 66.45)		
	Single	31	19 (61.3)	1.11 (0.01 - 52.00)	0.02	0.012
	Multiple	12	12 (100)	6.38 (0.56 - 66.45)		
Rural/Urban Category	Controls in:					
	urban areas	47	4 (8.5)	0.03 (0-2.69)	0.07	0.002
	rural areas	37	9 (24.3)	0.09 (0.01- 6.03)		
	Cases in:					
	urban areas	24	18 (75)	1.87 (0.01 - 66.45)	0.63	0.74
	rural areas	19	13 (68.4)	2.48 (0.01 - 30.05)		

[1]χ^2 test for proportion of sIgE sensitization;
[2]Mann-Whitney U / Kruskal-Wallis tests for IgE levels between single and multiple SPT cases as well as between urban and rural subjects in cases and controls.

to a frequency of over 4 times. The 3 subjects with frequencies over 4 times were all cases (8.6% of 35), compared to none of the 67 controls.

It is difficult to assess the prevalence of a positive SPT in the subjects with a claimed adverse response since we only tested 7 potential food allergens in the SPT. However, if we only consider the subjects with an adverse reaction to any of the food items that were tested in SPT, there were 11 out of 153 subjects (7.2%) with a positive SPT.

Very few children with a positive IgE response also presented adverse symptoms related to the specific food item. Only 3 out of 102 children for which both IgE and questionnaire data were available had adverse symptoms to the food to which they had a positive IgE titre (1 for pawpaw and 2 for pineapple).

Lifestyle Factors and SPT Reactivity to Food Allergens

Table 5 shows the effect of questionnaire-derived food consumption variables on the outcome of being a case. Although reported daily eating patterns were similar between cases and controls for most foods, we saw a significantly higher proportion of daily consumption of ice cream or yoghurt among cases (crude OR 4.2, 95% CI 1.1–15.6, p = 0.04). The same tendency was observed for daily rice consumption, though this did not reach statistical significance. The daily consumption of palm oil, on the other hand, was significantly lower among cases (crude OR 0.3, 95% CI 0.1–0.9, p = 0.04). None of the questionnaire-derived variables was independently associated with being a case after mutual adjustment.

The following reported early-life factors were not associated with being a case: premature birth, duration of exclusive breastfeeding, crèche or nursery attendance in the first 2 years of life, or the family's monthly expenditure on food. Body mass index (a surrogate for nutritional status) and current parasitic infections with malaria or helminths (hookworm, *Ascaris lumbricoides*, *Trichuris trichiura* or *S. haematobium*) were not associated with being a case. These results are shown in online supplementary Table S1.

Table 5: Associations between daily food habits and positive SPT for food.

Eating habit factors	N=89	Controls N=56	Cases N= 33	Crude OR (95% CI)	Adjusted OR (95% CI)
Daily consumption of dairy[1]	-	49 (87.5%)	24 (72.7%)	1.0	1.0
	+	7 (12.5%)	9 (27.3)	**4.2 (1.1 to 15.6)**	3.8 (0.9 to 16.3)*
Daily consumption of rice	-	28 (50%)	10 (30.2%)	1.0	1.0
	+	28 (50%)	23 (69.7%)	2.5 (0.9 to 6.5)	1.8 (0.6 to 5.1)
Daily consumption of palm oil	-	30 (53.6%)	26 (78.8%)	1.0	1.0
	+	26 (46.4%)	7 (21.1%)	0.3 (0.1 to 0.9)	0.3 (0.1 to 1.0)*

The adjusted odd ratios are corrected for age and all other variables,
ORs with p < 0.05 are in bold and *p <0.10
[1]Dairy refers specifically to ice cream or yoghurt.

Discussion

We assessed, for the first time in West Africa, the prevalence of allergic sensitization to local food allergens in children and how this relates to reported adverse reactions to foods in a school-based study. In contrast to the difference in prevalence between urban (with a higher prevalence of mite SPT in children from private schools) and rural (with a higher prevalence of cockroach SPT) children that we observed in this population (Obeng *et al*., manuscript in preparation), the prevalence of a positive SPT for food allergens was similar in urban and in rural children. Although we observed an overall tendency of an association between reported adverse reactions and SPT, this was significant only in urban children, and when specific food items were considered the association disappeared; this was probably due to lower statistical power. However, we have shown that there was a good agreement between the SPT and IgE antibody levels to specific foods in Ghanaian children, with a very strong association in urban children.

The overall prevalence of 5% skin test reactivity to any food in our population and between 0.3% and 2% for the specific food allergens is similar to and in some instances higher than reports from studies in other parts of the world [30]. The prevalence of reported adverse symptoms to peanut that we observed (1.5%) was a bit lower than that reported for children of similar age groups in the UK (1.9% for 6-year-old children, 1.8% for 11-year-old children, and 2.5% for 15-year-old children). Similarly, the percentage of children with a positive skin test for peanut was slightly lower in our study population (2.0%) compared to children in the UK (2.6–3.7%) [16, 31]. The prick-to-prick method allowed the testing of local foods, which would otherwise have been impossible with commercial extracts due to the poor sensitivity of such preparations. The panel of food allergens tested was selected from the local market and, with the exception of apple, they all form part of the regular Ghanaian diet. Our panel was limited to fresh fruits, aside from the commercial peanut extract, because they were readily available and did not require any previous preparation or cooking before consumption. Thus, it was easy to test the allergens in the same state as they are eaten. The strength of our study lies in its matched case-control design adjusting for demographic and environmental covariates which could be associated with atopy.

Even though in this study the panel of allergens included only 1 of the 8 major food allergens internationally recognized (peanut), it showed that other local foods may be of equal importance given the similar prevalence of sensitization to pineapple and peanut that we have found in this study. Dias *et al*. [25] found an increased prevalence of allergy to novel foods (i.e., foods which are not regularly used in food allergy test panels) like kiwi, legumes, and sesame, and they proposed that these may cause allergy mostly in the non-Caucasian population of the UK. Comparing skin test reactivity with IgE levels further demonstrated the importance of pineapple as an allergen as it showed the strongest association with sIgE antibody levels among cases stratified by specific food. None of the cases with a positive SPT to apple had elevated IgE levels. This is

interesting because it reflects other findings that have described differences between specific foods in the predictive value of food sIgE antibody levels for allergy [19]. It may also be due to the fact that apples are relatively new and less important in the diet of this population. It is also possible that sensitization to apple is due to cross-reactivity with another allergen either from a local pollen or from another food. It is not unlikely that this putative cross-reactive allergen is presented in the SPT but not in the CAP assay.

We also observed that the proportion of helminth infections was similar between cases and controls. This is important given that helminth infections are associated with an expansion of B cells producing IgE. Studies on atopy in Gabon have shown high mite sIgE levels in helminth-infected subjects who did not show skin reactivity to mites [32]. In our study, we observed among controls that helminth infection was associated with increased IgE levels to the tested foods. Also, controls from rural schools had significantly higher median sIgE levels than did controls from urban schools, an observation which could be explained by the prevalence of helminth infections in the rural schools. Limiting our analyses to rural subjects did not show current infection to be significantly associated with IgE levels in controls, suggesting that the general observation of higher IgE in rural controls could be due to a history of helminth infection.

Cross-reactivities between inhalant allergens and the plant food allergens used in our study have been reported to involve the protein profilin, a pan-allergen ubiquitously expressed in eukaryotic cells [33, 34]. In a recent study by Asero *et al*. [35], pollen-allergic patients who had a positive skin test reaction to date palm profilin were all sensitized to grass, and half of them had food allergy with oral symptoms. The offending foods included pineapple, citrus fruit, and banana. Similar results were reported earlier by Reindl *et al*. [36], when IgE reactivity to profilin was associated with adverse reactions to pineapple and banana. Previous data from Ghanaian schoolchildren showed a low prevalence, i.e. 0.3%, for grass pollen allergy (unpublished data). However, extracts from local flora would have to be included to be able to test the cross-reactivity to plants and/or trees prevalent in this region. Thus, our results with a prevalence of 5% could reflect sensitization specific to these tested plant foods and possibly independent of profilin or profilin sensitization via another local allergen.

Our results showed a higher proportion of multi-atopic cases with IgE sensitization to the specific foods eliciting skin reactivity than in cases atopic to single foods. Future studies could focus on the multi-atopic subjects to determine if being sensitive to some principal or ubiquitous allergen results in reactions to multiple food allergens. This would not only be useful in the management of food allergies resulting in symptoms, but would also prevent unnecessary dietary restrictions. It is also possible that a general allergic susceptibility could cause one to develop sensitization to a series of important inhalant and food allergens, which might be supported by our observation of a positive association between histamine wheal size (data not shown) and having a positive skin reaction to food allergens or aeroallergens; this finding has been previously reported by Van Gysel *et al*. [37] and de Bilderling *et al*. [38].

We also showed that the patterns of consumption of food were generally similar in cases and controls. However, the daily use of palm oil was significantly higher in controls while the daily consumption of ice cream, reflecting a less traditional but more contemporary diet as well as a higher SES, was higher in cases. Palm oil use may be associated with more traditional cooking which could prevent atopy. Though food consumption could be influenced by socioeconomic differences, the amount of money spent by the family monthly (a surrogate SES variable) or body mass index were not associated with being a case. In a recent study in schoolchildren in South Africa, an urban diet was associated with a positive SPT to aeroallergens, but there were no data on SPT to food [39].

We found a positive association between reported adverse reactions and skin test reactivity in the urban children, but only 20% of the subjects matched for the specific food item. It is known that reported food allergy reactions are not always reliable as indicators for food allergy and, indeed, reports from previous studies have indicated discrepancies between the reported perceived symptoms of food allergy and the results of clinical testing [EuroPrevall centres, van Ree, personal communication [7, 16]. The reported adverse reactions could indeed include intolerances and dislikes but, on the whole, they included food items internationally recognized as problematic common food allergens like egg, fish, and peanut. Notably, the reported symptom of tingling/itching of the mouth and/or lips, a reaction expected to be indicative of food allergy, was more frequently found in subjects with a food sensitization (positive SPT), although numbers were quite low (8.5% in SPT positive subjects vs. 2.3% in SPT negative subjects). Particularly interesting was the fact that avoidance of offending foods by subjects reporting an adverse reaction was indicated by 50% of the subjects, both in cases and in controls. The reason for this may be the absence of severe reactions to the foods. Reported adverse reactions were mainly gastrointestinal and respiratory in nature. While early-life factors like breastfeeding duration and birth weight are reported to be related to the development of allergy [40], we found no such association in this study; this could be due to small numbers.

This study provides timely information on the type and prevalence of sensitization to plant food allergens, the reported perceived symptoms of food allergy, and the relationship of these conditions with other lifestyle factors generally reported to be associated with allergy. Our results show that IgE-mediated food atopy is important in Ghana, potentially to a similar extent as in Europe and America. We also showed that in addition to peanut, a recognized major food allergen, pineapple is an important local allergen which merits further study in Ghanaian and foreign populations. Our study also shows that, similar to previous studies, perceived adverse reactions are not always reflected in SPT results and IgE antibody titres. These findings highlight the importance of further research into food allergy in Ghana and Africa as a whole. It will be useful in the future to look at the prevalence of the major food allergens documented in the US and Europe for a comparison with the local allergens. Such studies could also analyze the role of cross-reactivity and employ a more stringent gold standard of diagnosis like the DBPCFC to allow the determination of true clinical food allergy.

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Supplementary Material

Table S1: Associations between Lifestyle factors and positive SPT for food

Factors		Controls	Cases	Crude OR (95% CI)	Adjusted OR (95% CI)
Premature birth	-	57 (98.3%)	32 (94.1%)	1.0	1.0
	+	1 (1.7%)	2 (5.9%)	3.6 (0.3 to 40.8)	2.4 (0.1 to 71.6)
Exclusive breastfeeding for at least 3 months	-	25 (39.1%)	10 (29.4%)	1.0	1.0
	+	39 (61.9%)	24 (70.6%)	1.5 (0.6 to 3.8)	1.7 (0.4 to 6.5)
Day care attendance during first 2 years of life	-	47 (77.0%)	22 (62.9%)	1.0	1.0
	+	14 (23.0%)	13 (37.1%)	2.0 (0.8 to 4.9)	1.6 (0.4 to 1.0)
Expenditure on food per month (above median)	-	27 (28.2%)	17 (53.1%)	1.0	1.0
	+	29 (51.8%)	15 (46.9%)	0.8 (0.3 to 2.0)	0.5 (0.2 to 1.8)
Any helminth infection #	-	55 (76.4%)	27 (77.1%)	1.0	1.0
	+	17 (23.6%)	8 (22.9%)	1.0 (0.4 to 2.5)	0.9 (0.2 to 3.5)
Malaria infection	-	66 (79.5%)	33 (78.6%)	1.0	1.0
	+	17 (20.5%)	9 (21.4%)	1.1 (0.4 to 2.6)	0.8 (0.2 to 3.5)
BMI below normal $	-	64 (77.1%)	35 (81.4%)	1.0	1.0
	+	19 (22.9%)	8 (18.6%)	0.8 (0.3 to 1.9)	0.3 (0.1 to 1.9)

The adjusted odd ratios are corrected for age and all other variables.
Infection with any of the helminths: *S. haematobium*, *A. lumbricoides*, *T. trichiura* or hookworm.
$ Weight categories are as described in Hogewoning AA *et al. British Journal of Dermatology.* 2009; 161 (2): 475-7.
Odds ratios with $p < 0.05$ are in bold.


NET WEIGHT 50 KGS
ORIGIN : THAILAND
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chapter 4

Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity

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Abstract

Background: The prevalence of peanut allergy has increased in developed countries, but little is known about developing countries with high peanut consumption and widespread parasitic infections.

Objective: We sought to investigate peanut allergy in Ghana.

Methods: In a cross-sectional survey among Ghanaian schoolchildren (n = 1604), data were collected on reported adverse reactions to peanut, peanut sensitization (serum specific IgE and skin reactivity), consumption patterns, and parasitic infections. In a subset (n = 43) IgE against Ara h 1, 2, 3, and 9 as well as cross-reactive carbohydrate determinants (CCDs) was measured by using ImmunoCAP. Cross-reactivity and biological activity were investigated by means of ImmunoCAP inhibition and basophil histamine release, respectively.

Results: Adverse reactions to peanut were reported in 1.5%, skin prick test reactivity in 2.0%, and IgE sensitization (≥0.35 kU/L) in 17.5% of participants. Moreover, 92.4% of those IgE sensitized to peanut (≥0.35 kU/L) had negative peanut skin prick test responses. *Schistosoma haematobium* infection was positively associated with IgE sensitization (adjusted odds ratio, 2.29; 95% CI, 1.37-3.86). In the subset IgE titres to Ara h 1, 2, 3, and 9 were low (<1.3 kU/L), except for 6 moderately strong reactions to Ara h 9. IgE against peanut was strongly correlated with IgE against CCDs ($r = 0.89$, $p < 0.001$) and could be almost completely inhibited by CCDs, as well as *S. haematobium* soluble egg antigen. Moreover, IgE to peanut showed poor biological activity.

Conclusions: Parasite-induced IgE against CCDs might account largely for high IgE levels to peanut in our study population of Ghanaian schoolchildren. No evidence of IgE-mediated peanut allergy was found.

Clinical Implications

Peanut-specific IgE antibodies in Ghana, a Sub-Saharan African country, show cross-reactivity with clinically irrelevant carbohydrate determinants and therefore may reduce the diagnostic value of this parameter in establishing peanut allergy.

Capsule Summary

In Ghana where peanut consumption is high and parasitic infections widespread, elevated peanut-specific IgE levels may primarily be due to cross-reactive carbohydrate determinants and may not result in skin reactivity or reported symptoms.

Key Words

Peanut allergy, skin prick testing, Immunoglobulin E, Sub-Saharan Africa, IgE cross-reactivity, cross-reactive carbohydrate determinants, helminth infections, basophil histamine release, EuroPrevall

Abbreviations

adjOR: Adjusted odds ratio
AWA: Adult worm antigen
BHR: Basophil histamine release
CCD: Cross-reactive carbohydrate determinant
CI: Confidence interval
CRD: Component-resolved diagnostics
SEA: Soluble egg antigen
SPT: Skin prick test

Introduction

Recent studies report a significant rise in the incidence of peanut allergy particularly in Europe and North America where self-reported peanut allergy is around 1% among individuals less than 18 years [1, 2]. According to a 5 year follow-up survey among children in Montréal, Canada, peanut allergy prevalence (confirmed by skin prick tests and oral food challenges) rose from 1.34% in 2000-2002 to 1.62% in 2005-2007 [3] while a population-based study conducted in Australia among infants aged 12 months found the prevalence of challenge-proven peanut allergy to be 3.0% [4].

Although extensive peanut allergy research has been conducted in Western countries, there are only a few published studies from other areas of the world where peanut consumption is high such as in South-East Asia. A population-based questionnaire survey in children 4-6 years as well as 14-16 years in two Asian populations indicates that self-reported adverse reactions to peanut in this region may vary between 0.43% and 0.64% [5]. Additionally, a food allergy study among children 6-11 years in China, India and Russia described peanut allergy to be uncommon in all three countries [6]. For Sub-Saharan Africa, no published data are available to date.

One reason proposed to explain the lower prevalence of allergic disorders in many developing countries is the possible suppressive role of chronic infections on the development of allergies [7]. Infections, especially parasitic ones, are highly prevalent in Africa, Asia and South America, particularly in rural areas or in poor sections of urban communities [8-10]. One mechanism by which helminth infections are believed to protect against allergies is by activating regulatory networks that involve the induction of regulatory T and B cells as well as the modulation of innate immune cells [11, 12]. Another mechanism of recent interest has been how cross-reactivity between parasite/helminth antigens and allergens may affect IgE sensitization patterns and their translation into clinical symptoms [13, 14].

As there is little information on peanut allergy in Sub-Saharan Africa and on associated risk factors, we set out to investigate the epidemiology of peanut allergy in schoolchildren in Ghana, a country where peanut consumption is estimated to be high. In 2009 alone, the per capita consumption of peanuts in Ghana was approximately 12 kg [15] compared to a per capita estimate of 6.6 kg for the United States in the same year [16]. Our objective was to identify factors associated with peanut sensitization and reported symptoms such as parasitic infections, peanut consumption patterns and peanut preparation methods. We also sought to characterize IgE reactivity to peanut in our population.

Methods

Study design and population

We conducted a cross-sectional study between March 2006 and March 2008 that was part of a larger investigation into allergic sensitization and parasitic infections in schoolchildren in Southern Ghana. This investigation was carried out within the

framework of the European Union-funded EuroPrevall [17] and GLOFAL [18] projects (see details in the supplementary material). Outcome parameters of interest were 1. reported adverse reactions to peanut and 2. peanut sensitization based on serum specific immunoglobulin E (IgE) levels and skin prick test (SPT) reactivity. The study was approved by the Noguchi Memorial Institute for Medical Research Institutional Review Board, Ghana (NMIMR-IRB CPN 012/04-05). Three districts in the Greater Accra Region were selected for the investigation. Within these districts, schools were randomly selected and approached to participate in the study (see sampling methodology in the supplementary material).

We recruited children aged between 5 and 16 years attending 6 rural and 3 urban schools. Approximately 35% (1714/4852) of all children attending targeted schools agreed to participate in the study (see Figure E1 in the supplementary material). The overall participation rate in the rural schools was 34.7% compared to 36.4% in the urban schools. There was no information available on non-participants. Of 1714 children enrolled, 59 subjects were in the end unavailable for data collection while 51 were excluded for being outside of the age-range (see Figure E2 in the supplementary material), leaving a total study population of 1604 children. Parameters measured were IgE serology (n=1328), skin prick test reactivity (n=1396), questionnaire (n=1372), urinary schistosomiasis (n=1537), intestinal helminths (n=1398) and malaria blood films (n =1468).

Component-resolved diagnostics (CRD) could only be performed for a maximum of 50 subjects due to budgetary limitations. Subjects for whom a sufficient serum volume (≥350 µL) was available were included based on reported adverse reactions to peanut (n=8), peanut SPT positivity (n=15) and randomly selected subjects with IgE to peanut levels higher than 1.5 kU/L (n=15). This threshold was chosen to increase the sensitivity for measuring IgE against individual peanut allergens. Five randomly selected negative control subjects with no reported adverse reactions to peanut and no peanut sensitization were also included. Detailed selection procedure for the CRD subset can be found in the supplementary material.

Parasitological examinations

One stool sample per subject was collected for the detection of intestinal helminth eggs by the Kato-Katz technique [19] using 25 mg of stool. A urine sample was also collected to determine *S. haematobium* infection using the standard filtration method [20] in which 10 ml of urine is filtered through a nylon nucleopore filter (pore size, 12 µm). For each subject, a small quantity of blood was collected to prepare a Giemsa-stained thick smear slide to detect malaria.

Questionnaire

A standard questionnaire (see thesis appendix) was administered to the parents or guardians of study subjects to collect information on demographic and socioeconomic parameters as well as information on established risk factors for the development

of allergy. Questions on the symptoms of adverse reactions to food were included in the questionnaire. These were adapted from the validated EuroPrevall survey questionnaire [21]. The questionnaire was administered by trained interviewers who were fluent in the local language of each participant. It was pre-tested in a pilot study under field conditions to ensure understanding and acceptability.

Skin prick testing

Skin prick test reactivity to a commercially available whole peanut extract (kindly provided by ALK-Abelló, Madrid, Spain) was assessed using the standard protocol [22, 23] as has been described in detail elsewhere [24]. We defined peanut SPT positivity as a mean wheal diameter ≥ 3 mm [25].

IgE antibody measurements

ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) measurements were carried out following the manufacturer's instructions. IgE to peanut was assessed in all participants and 0.35 kU/L was used as the sensitization cut-off. A cut-off of ≥15 kU/L, that is reported to have a positive predictive value of 95% for clinical peanut allergy [26], was also examined.

For the CRD subset (n=43), specific IgE to recombinant peanut allergens (rAra h 1, 2, 3 and 9), profilin (rPhl p 12) and to bromelain, a marker for cross-reactive carbohydrate determinants (CCD), was assessed by ImmunoCAP. Bet v 1 homologous Ara h 8 was excluded from the analysis because there is no exposure to Fagales tree pollen in Ghana.

IgE inhibition assays

Titrated ImmunoCAP inhibition assays were conducted to establish the degree of cross-reactivity of peanut-specific IgE. To this end, 75 µL of pooled serum comprised of equal volumes of 17 sera (all with peanut-specific IgE levels ≥5.5 kU/L and similar IgE responses to peanut as well as to bromelain) was mixed with 75 µL of inhibitor. Inhibitors used were either bromelain, *Schistosoma haematobium* soluble egg antigen (SEA), *Schistosoma haematobium* adult worm antigen (AWA) or *Ascaris lumbricoides* antigen. For three subjects, two with high and one with low IgE titres to Ara h 9, individual sera were also tested by ImmunoCAP inhibition. Each serum pool (or individual sera) was pre-incubated with an inhibitor at room temperature for 1 hour. Subsequently, samples were analysed for peanut-specific IgE as described above. Results were expressed as percentages of an uninhibited control (phosphate buffered saline).

Basophil histamine release (BHR) assays

To assess the biological activity of peanut-specific IgE in our population, BHR assays were performed using stripped basophils from a non-allergic donor that were sensitized with sera of subjects selected from the CRD subset (n=43). Two sera with similar levels

of IgE against peanut and CCD were selected. In addition, two with higher IgE against peanut than against CCD in combination with high IgE against Ara h 9 were also evaluated (see full characteristics in Table E1 in the supplementary material). BHR assays were performed as has been described elsewhere [27, 28].

Statistical analysis

Analysis was carried out using STATA version 10 (StataCorp, Texas, USA). Urban-rural differences in subject characteristics as well as in peanut sensitization (IgE and SPT) and reported adverse reactions were examined by Pearson's χ^2 tests (with 1 degree of freedom). To assess factors associated with peanut sensitization (IgE and SPT) and reported adverse reactions, multivariable random effects logistic regression models were fitted that took into account possible correlation among observations within each school by modelling school as a random effect. This approach was used since children attending the same school were likely to share common characteristics as well as exposures. Models were adjusted for age, sex and urban-rural area (as *a priori* confounders) along with other variables significant from crude analysis.

Results

Characteristics of the study population

The characteristics of the study participants stratified by area are given in Table I. There were no significant differences in gender distribution and age-group comparing the two areas although urban children had a slightly higher median age. In addition, rural subjects had significantly more helminth infections and malaria.

Although peanut consumption was high in both areas, reported daily consumption was considerably higher among rural schoolchildren (36.2%) compared to their urban counterparts (9.8%). Furthermore, in the rural area, both "boiled only" and "roasted only" peanut preparation methods were reported more frequently than in the urban area where the combination of roasting and then boiling peanuts in soup preparation was more common. Topical exposure to peanut as assessed by the use of peanut oil as a skin ointment was higher in rural compared to urban schools.

Reported adverse reactions and sensitization (IgE and SPT) to peanut

Adverse reactions were reported in 1.5% (n=21/1372) of participants (see Table II) most of whom were rural schoolchildren. The distribution pattern of the characteristics of those reporting adverse reactions (see Table E2 in the supplementary material) did not differ significantly from the rest of the study population (statistical tests data not shown). About 67% of those reporting adverse reactions to peanut had gastrointestinal complaints and 43% had complaints described as itching of the mouth or difficulty swallowing. Only 4 out of 21 subjects reported a reaction time "within minutes" (see Table E3 in the supplementary material).

Table I. Characteristics of study population stratified by area

FACTOR		ALL n / N (%)	AREA		
			Rural n / N (%)	Urban n / N (%)	P-value #
Sex					
Males		757 / 1604 (47.2)	465 / 976 (47.6)	292 / 628 (46.5)	0.65
Females		847 / 1604 (52.8)	511 / 976 (52.4)	336 / 628 (53.5)	
Age					
<11 years or less		785 / 1604 (48.9)	496 / 976 (50.8)	289 / 628 (46.0)	0.06
≥more than 11years		819 / 1604 (51.1)	480 / 976 (49.2)	339 / 628 (54.0)	
Parasitic Infections					
Any intestinal helminth §	(positive)	248 / 1398 (17.7)	236 / 834 (28.3)	12 / 564 (2.1)	**<0.001**
S. haematobium	(positive)	103 / 1537 (6.7)	83 / 922 (9.0)	20 / 615 (3.3)	**<0.001**
*Plasmodium species**	(positive)	349 / 1468 (23.8)	310 / 880 (35.2)	39 / 588 (6.6)	**<0.001**
Peanut Consumption					
Daily	(yes)	365 / 1372 (26.6)	316 / 874 (36.2)	49 / 498 (9.8)	**<0.001**
Weekly	(yes)	760 / 1372 (55.4)	438 / 874 (50.1)	322 / 498 (64.7)	**<0.001**
Monthly	(yes)	183 / 1372 (13.3)	70 / 874 (8.0)	113 / 498 (22.7)	**<0.001**
Every 6 months	(yes)	21 / 1372 (1.5)	12 / 874 (1.4)	9 / 498 (1.8)	0.52
Never	(yes)	35 / 1372 (2.6)	35 / 874 (4.0)	0 / 498 (0.0)	**<0.001**
Missing Consumption information		8 / 1372 (0.6)	3 / 874 (0.3)	5 / 498 (1.0)	
Exclusive Peanut Preparation Methods					
Boiled ONLY	(yes)	61 / 1372 (4.4)	56 / 874 (6.4)	5 / 498 (1.0)	**<0.001**
Fried ONLY	(yes)	19 / 1372 (1.4)	19 / 874 (2.2)	0 / 498 (0.0)	**0.001**
Roasted ONLY	(yes)	277 / 1372 (20.2)	276 / 874 (31.6)	1 / 498 (0.2)	**<0.001**
Other Peanut Preparation Methods					
Raw	(yes)	22 / 1372 (1.6)	3 / 874 (0.3)	19 / 498 (3.8)	**<0.001**
Peanut Oil**					
Use of peanut oil	(yes)	33 / 1370 (2.4)	32 / 872 (3.7)	1 / 498 (0.2)	**<0.001**

P-values were calculated by using Pearson's χ^2 test (1 degree of freedom). Values in boldface indicate significance.
§ Any intestinal helminth= *Ascaris lumbricoides*, hookworm (*Ancylostoma duodenale* or *Necator americanus*), *Trichuris trichiura* or *Schistosoma mansoni*.
* *Plasmodium* species = *Plasmodium falciparum* or *Plasmodium malariae* (the 2 malaria parasite species detected in our study population).
** Peanut oil use information missing for 2 participants.

The percentage of subjects with a positive peanut SPT was 2.0% (n=28/1396) and this was not significantly different between the two areas (see Table II). Positive wheal sizes for peanut ranged from 3.0 mm to 6.5 mm and did not vary between areas (data not shown).

Peanut IgE sensitization (≥0.35 kU/L) was observed in 17.5% (n=233/1328) of the study population with 23.6% of rural children being sensitized compared to 9.7% of

Table II. Prevalence of adverse reactions to peanut and peanut sensitization (SPT and IgE) stratified by area

		AREA		
FACTOR	**ALL n / N (%)**	**Rural n / N (%)**	**Urban n / N (%)**	**P value #**
Adverse reactions to food				
Any food	154 / 1372 (11.2)	115 / 874 (13.2)	39 / 498 (7.8)	**0.003**
Peanut	21 / 1372 (1.5)	18 / 874 (2.1)	3 / 498 (0.6)	**0.035**
Skin prick test reactivity				
Peanut Positive	28 / 1396 (2.0)	17 / 881 (1.9)	11 / 515 (2.1)	0.79
Peanut-specific IgE				
≥0.35 kU/L	233 / 1328 (17.5)	177 / 751 (23.6)	56 / 577 (9.7)	**<0.001**
≥15 kU/L	12 / 1328 (0.9)	8 / 751 (1.1)	4 / 577 (0.7)	0.48

P-values were calculated by using Pearson's χ^2 test (1 degree of freedom).
Values in boldface indicate significance.

urban participants (p <0.001). However, 92.4% (n=194/210) of those IgE sensitized to peanut (≥0.35 kU/L) were peanut SPT negative. Interestingly, 0.9% (n=12/1328) of the study subjects were highly sensitized when using the IgE cut-off of ≥15 kU/L, which is reported to have a positive predictive value of 95% for clinical peanut allergy [26], but only 1 of them reported reactions. Figure 1 shows the overlap between the peanut-related outcomes for study subjects with complete allergy data (reported reactions, SPT and IgE). No individual was positive for all three parameters.

Factors associated with peanut sensitization (IgE and SPT) and reported adverse reactions to peanut

In multivariable analysis, area was strongly associated with peanut IgE sensitization ≥0.35 kU/L with urban subjects having a reduced odds of elevated IgE relative to their rural counterparts [adjOR= 0.41, 95% CI (0.25 - 0.67), p <0.001] (see Table III). Being *S. haematobium* infected was also associated with peanut IgE sensitization [adjOR= 2.29, 95% CI (1.37 - 3.86), p <0.001] while intestinal helminth infection was not.

Although the majority of peanut IgE sensitized individuals were not peanut SPT positive, almost all peanut SPT positive subjects were IgE sensitized. Thus, in multivariable analysis, IgE sensitization was associated with peanut SPT reactivity [adjOR= 17.09, 95%CI (6.30 – 46.36), p <0.01]. In addition, while not observed in crude analysis, residing in the urban area was associated with a significantly higher chance of being SPT positive to peanut after adjusting for confounders (see Table III). No other factors, including helminth infection, had an effect on SPT to peanut (see Table III).

Data on peanut consumption and preparation methods as risk factors for peanut-related outcomes are shown in Table E4 (see supplementary material). 'Never' consuming peanuts, as a proxy for avoidance, was associated with reported symptoms [adjOR=5.40,

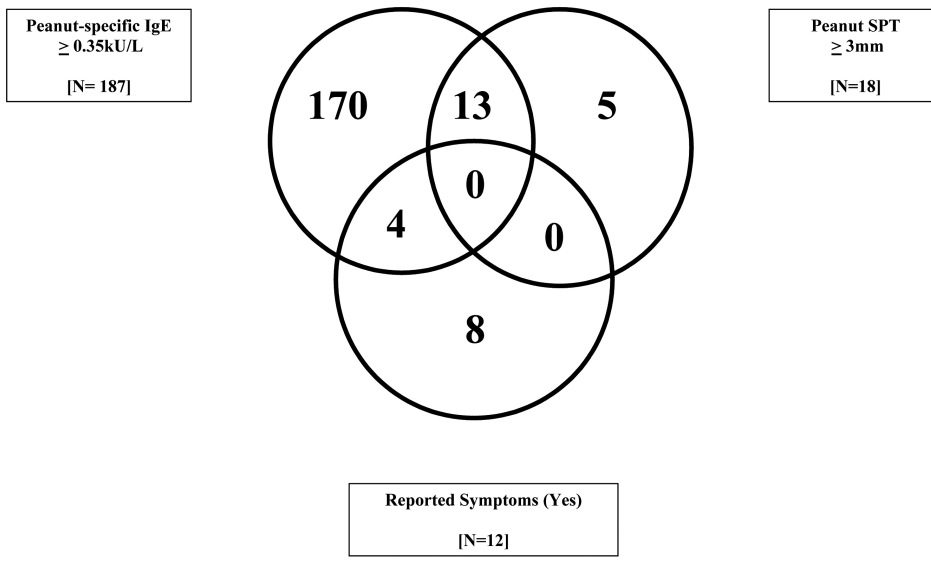

Figure 1: Overlap between peanut allergy outcomes
Overlap between reported adverse reactions to peanut and peanut sensitization (IgE levels and SPT responses) for subjects with complete data for allergy-related parameters. (n=1004).

95% CI (1.47 – 19.80), p <0.05]. Raw peanut consumption was also linked to reported adverse reactions to peanut [adjOR=17.14, 95% CI (2.93 – 100.45), p <0.01]. However, numbers were low as reflected in the wide confidence interval. All other factors, including helminth infection, were not significantly associated with reported adverse reactions to peanut (see Table III and Table E4 in the supplementary material).

Component-resolved IgE testing

Figure 2A shows the results of CRD performed in a subset (n=43) to better characterize peanut-specific IgE. Those with IgE to peanut >1.5 kU/L (median 12.5 kU/L) had high levels of IgE to CCD but low IgE responses (<1.3 kU/L) to rAra h 1-3 and rPhl p 12. A strong correlation was seen between peanut-specific IgE and CCD-specific IgE [r = 0.89, p <0.001] (see Figure 2B). For some individuals, IgE against peanut was significantly higher than to CCD and in 6 of these, high titres of IgE to the lipid transfer protein rAra h 9 were observed (see Figure 2A). Of note, 4 out of 6 of these subjects were peanut SPT positive (see Table E1 in the supplementary material).

Inhibition of IgE binding to peanut by CCD and schistosome egg antigen

Titrated CAP-inhibition assays demonstrated that binding of IgE from a serum pool of individuals (n=17) with similar IgE titres to peanut as to CCD was almost completely

Table III. Factors associated with reported adverse reactions to peanut and peanut sensitization (IgE and SPT)

	Peanut-Specific IgE (≥ 0.35ku/l vs. <0.35ku/L)		Peanut SPT Positive (+ vs -)		Reported Adverse Reactions to Peanut (Yes vs. No)	
Factors	**Adjusted OR (95%CI)**	**Wald's Test P-value**	**Adjusted OR (95% CI)**	**Wald's Test P-value**	**Adjusted OR (95% CI)**	**Wald's Test P-value**
Peanut-Specific IgE (≥ 0.35 kU/L vs. < 0.35 kU/L)			**17.09 (6.30 - 46.36)**	**<0.001**	1.94 (0.57 – 6.63)	0.29
Peanut SPT Positive (+ vs -)					2.82 (0.35 – 22.70)	0.33
Age (≥11 years vs < 11 years)	1.07 (0.78 – 1.47)	0.67	1.36 (0.55 – 3.36)	0.51	0.58 (0.24 – 1.42)	0.23
Gender (Male vs Female)	1.12 (0.83 – 1.51)	0.47	1.65 (0.67 – 4.03)	0.27	0.68 (0.28- 1.65)	0.39
Area (Urban vs. Rural)	**0.41 (0.25 – 0.67)**	**<0.001**	**2.94 (1.03 – 8.40)**	**0.044**	0.30 (0.09 – 1.01)	0.052
Any intestinal helminth § (+ vs -)	1.01 (0.66 – 1.55)	0.97	0.69 (0.17 – 2.84)	0.61	0.35 (0.08 - 1.56)	0.17
S. haematobium (+ vs -)	**2.29 (1.37 – 3.86)**	**0.002**	0.41 (0.05 – 3.42)	0.41	0.65 (0.08 – 4.95)	0.67
*Plasmodium species** (+ vs -)	1.10 (0.77 – 1.56)	0.61	0.49 (0.13 – 1.82)	0.28	0.59 (0.16 – 2.20)	0.44

Peanut-specific IgE models were adjusted for age, sex, area and *S. haematobium* infection.
Peanut SPT models were adjusted for age, sex, area and peanut-specific IgE.
Reported peanut reaction models were adjusted for age, sex and area.
§ Any intestinal helminth= *Ascaris lumbricoides*, hookworm (*Ancylostoma duodenale* or *Necator americanus*), *Trichuris trichiura* or *Schistosoma mansoni*.
* *Plasmodium* species = *Plasmodium falciparum* or *Plasmodium malariae* (the 2 malaria parasite species detected in our study population).
Values in boldface indicate significance.

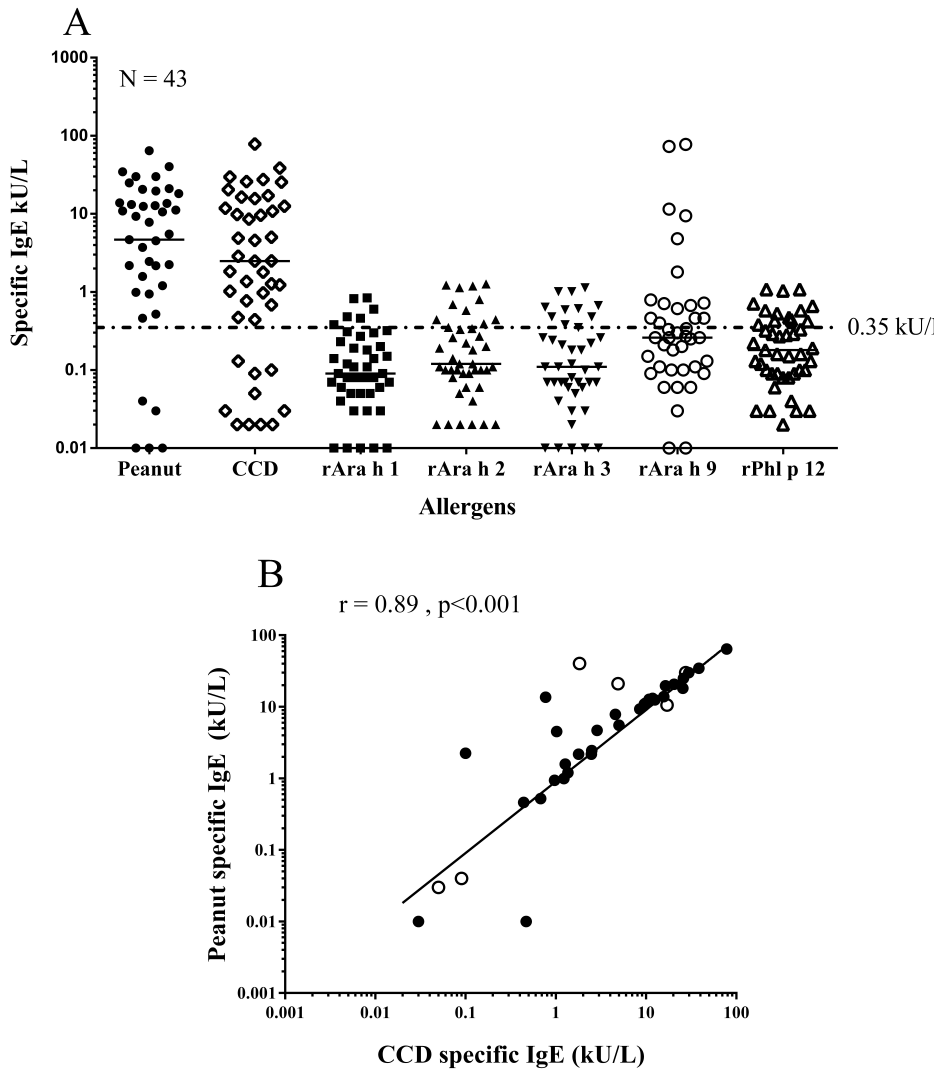

Figure 2: Characterization of specific IgE to peanut in a subset

[A] Measurement of specific IgE to whole peanut extract, recombinant peanut allergens, profilin and CCD marker bromelain in a subset (n=43). Median specific IgE levels are indicated by black lines. The dotted line shows IgE sensitization cut-off of 0.35 kU/L. **[B]** Correlation between peanut-specific IgE and CCD-specific IgE. Open circles (O) indicate subjects with IgE to rAra h 9 of greater than 1.5 kU/L.

inhibited by CCD as well as by *S. haematobium* SEA (see Figure 3). Individual inhibitions for two subjects with high IgE to peanut and to Ara h 9 as well as low IgE to CCD showed <10% inhibition by SEA (see Table E1 in the supplementary material). In addition, *S. haematobium* AWA and A. lumbricoides antigen did not inhibit binding significantly (data not shown).

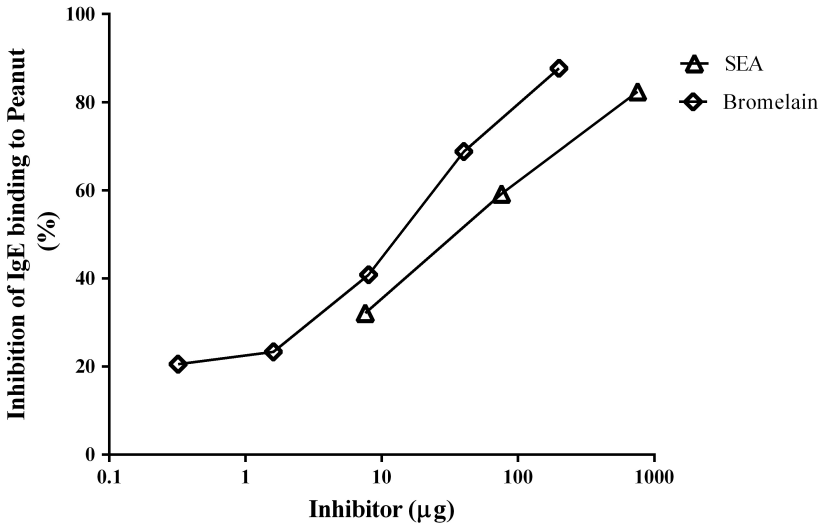

Figure 3: Inhibition of IgE binding to peanut

Inhibition of IgE binding to whole peanut by bromelain and *S. haematobium* soluble egg antigen (SEA) by using pooled sera (n=17). The figure shows that IgE binding to whole peanut extract was almost completely inhibited by bromelain (◊) and *S. haematobium* soluble egg antigen (Δ) respectively.

BHR Assays

Peanut extract induced little histamine release when basophils were sensitized with IgE from subjects with similar IgE reactivity to peanut as to CCD (see Figure 4A and 4B). For these individuals, the ability of *S. haematobium* SEA to induce histamine release was tested and only at a concentration of 10 µg/ml, release was observed. For two subjects with titres of IgE against Ara h 9 >70 kU/L (see Figure 4C and 4D), Arah 9 induced significant histamine release starting at 10 pg/ml reaching maximum release at about 1 ng/ml while with peanut extract, release was seen starting from a concentration of 10 µg/ml.

Discussion

Our study is the first investigation of reported adverse reactions to peanut and peanut sensitization based on serum specific IgE as well as SPT reactivity in Sub-Saharan Africa among an unselected group of children. We confirmed that there was a high frequency of daily peanut consumption in Southern Ghana particularly among rural schoolchildren. We also observed an association between reported peanut adverse reactions and peanut avoidance. The percentage of reported peanut adverse reactions among schoolchildren in our survey was 1.5%. However, the majority of these reported reactions occurred within hours/days while IgE-mediated peanut allergy is typically associated with symptoms appearing within minutes or up to 2 hours [29].

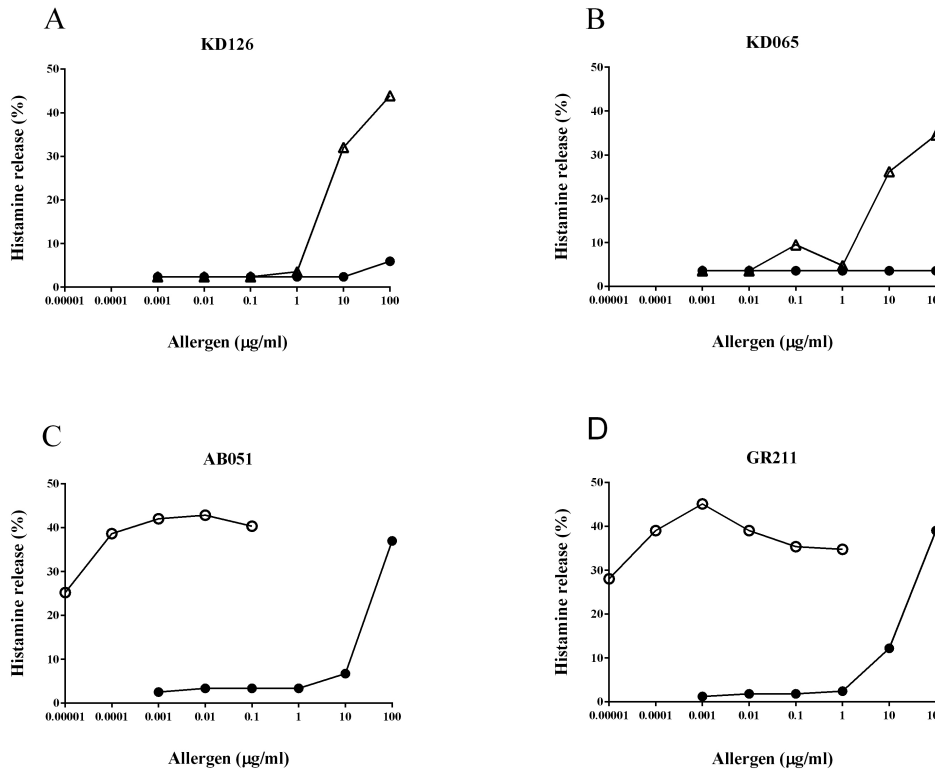

Figure 4: Basophil histamine release assays

Basophil histamine release assay results. Basophil histamine release induced by peanut extract (●), *S. haeamatobium* SEA (Δ) and Ara h 9 (O). **[A]** and **[B]** are results for two subjects with high IgE titres against peanut and CCD. **[C]** and **[D]** are results for two subjects with high IgE titres against peanut and Ara h 9 but low IgE titres against CCD.

Among study participants, 2.0% were peanut SPT positive. Although 17.5% of all subjects had elevated IgE to peanut (≥0.35 kU/L), 92.4% of these were peanut SPT negative. One explanation for the discrepancy between specific IgE and SPT could be the suppression of IgE induced inflammation by immunological regulatory networks [30] that might be operative during chronic helminth infections. However, we did not observe any association between helminth infection and SPT to peanut.

Notably, 12 out of 1328 participants had peanut-specific IgE levels ≥15 kU/L; a cut-off reported to have a positive predictive value of 95% for clinical peanut allergy in a European study population [26] but was virtually unaccompanied by reported symptoms in our study. This highlights the limitations in applying cut-off values determined in one population to other populations.

Analysis by component-resolved diagnostics in a subset indicated that the majority of those with high titres of IgE against peanut (median 12.5 kU/L) had low responses

(<1.3 kU/L) against the major peanut allergens (Ara h 1, 2 and 3) commonly associated with peanut allergy [31-33]. Recently, IgE responses to Ara h2 in particular, have been used to differentiate between clinical peanut allergy and asymptomatic peanut sensitization [34] as well as to improve diagnostic accuracy [35]. One study observed that a cut-off of IgE to rAra h2 >0.23 kU/L had a specificity of 97% and sensitivity of 93% among peanut allergic patients and controls in France [32]. Taken all together, sensitization to peanut storage proteins in Ghana appears weak and rare compared to European or US peanut allergic patients. The lack of clinical reactivity among study participants with elevated IgE responses to Ara h 2 would have to be explored further.

The most dominant molecular component recognized by IgE in peanut-sensitized subjects in our subset was CCD. A strong correlation was observed between IgE to peanut and to CCD. CCDs are N-glycans in plants and invertebrate glycoproteins that result in a high degree of cross-reactivity between pollen and foods [36]. CCDs have negligible *in vivo* biological activity as well as clinical relevance [37-39]. Grass pollen was found to be of minor importance in Ghanaian schoolchildren as was established in a pilot study preceding the present survey. In our study population, we observed that current *S. haematobium* infection was associated with elevated IgE to peanut. Moreover, among our subset, the results of the ImmunoCAP inhibition assays showed that plant-derived CCD (bromelain) inhibited IgE binding to peanut and that a *Schistosoma*-derived glycoprotein preparation was an equally potent inhibitor. These observations suggest that carbohydrate specific IgE is induced by glycoproteins from the eggs of *S. haematobium* that are different from but cross-reactive with those on bromelain. Interestingly, *Schistosoma* adult worm glycoproteins were not effective as inhibitors indicating the importance of stage-specific N-glycans in this cross-reactivity. The importance of cross-reactivity might also explain the residual effect for rural area on IgE to peanut, which was seen after adjusting for current *S. haematobium* infection. Past infections in individuals residing in the rural area might have led to cross-reactive IgE to peanut.

Interestingly, in the studied subset, IgE responses to Ara h 9 were elevated in 6 children with two having IgE titres >70 kU/L. Furthermore, IgE antibodies against Ara h 9 were biologically active at low allergen concentrations (pg/ng range) as determined by basophil histamine release assays. The observation that 4 out of 6 subjects with high IgE to Ara h 9 were peanut SPT positive is in line with these BHR results. However, none of these reported immediate adverse reactions to peanut. Altogether, the data suggest that sources other than CCD could contribute to elevated IgE to peanut extract. The origin of sensitization to this lipid transfer protein is unknown and whether a locally consumed fruit is at the basis of this sensitization, as is commonly reported in Europe in relation to peach [31, 40, 41], remains to be determined for Ghana.

Our study had a number of limitations such as a low participation rate but given our observation that IgE-mediated peanut allergy in Ghanaian schoolchildren is rare (if existing at all), it is unlikely that selection bias is affecting our findings in this respect. However, the borderline significant difference in age between rural and urban children

as well as the fact that the rural population is from areas that are endemic for helminth infections need to be taken into account when considering the generalizability of our findings. The absence of the gold standard for peanut allergy (oral food challenges) is another limitation but given that reported adverse reactions to peanut were largely not accompanied by immediate reactions, this is less likely to be an issue. An additional study weakness is the use of a questionnaire as a measurement tool for adverse reactions as well as other self-reported parameters. Furthermore, our school-based study design meant that children less than 5 years were excluded from the investigation which might bias the results by omitting an important age-group affected by peanut allergy. However, given the persistent nature of peanut allergy among most individuals, the effect of an older age cut-off of 5 years is likely to be minimal. The fact that CRD was conducted in a relatively small subset of our larger study population is another limitation although the subset did not differ from the wider study population on key demographic factors and parasitic infections.

Despite these limitations, our study provides new insights into the nature of peanut sensitization and reported adverse reactions to peanut in Ghana, a Sub-Saharan African country where peanut consumption is high but does not appear to translate into true peanut sensitization, let alone peanut allergy. Overall, our observations suggest that IgE-mediated peanut allergy in Ghanaian schoolchildren is rare. Among a subset, we found a role for N-glycans, particularly related to *Schistosoma*, in inducing cross-reactivity resulting in elevated IgE to peanut without skin reactivity or reported symptoms. This study once more highlights the poor biological activity of CCD-specific IgE. Interestingly, IgE to Ara h 9 demonstrated normal biological activity suggesting that lack of biological activity is not the only explanation for the lack of clinical peanut allergy. Future studies on the characteristics of cross-reactive IgEs and the pathways behind their development may be essential to the ongoing investigation of immune regulatory mechanisms in an effort to curtail strong allergic inflammation.

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Supplementary material

Study overview: EuroPrevall and GLOFAL

We conducted a cross-sectional investigation that was carried out within the framework of the European Union-funded EuroPrevall and Global View of Food Allergy (GLOFAL) projects. EuroPrevall was a multi-disciplinary project examining the prevalence, cost and basis of food allergy in Europe that ran between June 2005 and December 2009 [E1].

It involved the collaboration of 17 European Union member states as well as non-European partners [E2]. The main focus of the project was to explore the patterns and the prevalence of food allergies across Europe as well as to improve the diagnosis of food allergy. Within the frame of the EuroPrevall Project, the multi-centre GLOFAL initiative was formulated to provide insights from developing countries in Africa (Gabon and Ghana) as well as Asia (Indonesia). GLOFAL was a collaborative project between researchers in developing countries and Europe-based EuroPrevall partners [E3]. One of the specific objectives of the GLOFAL project was to generate novel insights into the interaction between food consumption, the immune system and the development of allergies. It was ultimately expected that the identification of risk factors for allergy in rapidly urbanizing countries may prevent allergy epidemics in these areas while the identification of protective factors may be useful in stemming the allergic march in more industrialized parts of the world [E4].

Sampling Methodology

For our investigation, a school-based design was deemed to be the most logistically feasible approach. To this end, urban and rural schools were targeted. Of particular interest were areas where parasitic infections were known to be prevalent and where no school-based mass deworming exercises had been conducted in recent years.

Out of the 10 administrative regions of Ghana, the Greater Accra Region was selected for the study. This was in part because the host institute for the research project, the Noguchi Memorial Institute for Medical Research was located in this region. In addition, areas within the Greater Accra region remain endemic for helminth infections and malaria. In 2006, the Greater Accra Region was comprised of 6 districts 2 of which were urban (Accra Metropolis and Tema Municipal Area). Out of the 4

remaining largely rural districts, we targeted ones where no district-wide school-based mass deworming program had taken place in recent years. Therefore, the Ga East and Dangme East districts were selected. Within both districts, one sub-district was randomly selected. Lists of all schools in the targeted sub-districts were obtained and each school approached about willingness to participate in the study.

For our urban schools, we selected one of the two urban districts of the Greater Accra Region, the Accra Metropolis. Out of the 6 sub-metros of Accra, two were selected. Within the two sub-metros, a list of all private and public schools with an enrolment greater than 200 that were also located within a 10 km radius of the host institute was generated. All schools were approached about willingness to participate in the study. After a school head agreed to participate, meetings were held where details of the study were verbally explained to parents with the aid of a powerpoint presentation. These meetings were conducted in the appropriate local language and information sheets distributed. Once a parent or guardian agreed to enrol their ward(s), signed or thumb-printed individual informed consent forms were obtained for each verbally assenting study subject. Schools where study enrolment was >30% were included in our analysis.

Selection of Component-Resolved Diagnostics (CRD) subset

Having observed that a significant proportion of study participants had elevated IgE to peanut without skin prick test reactivity and reported adverse reactions to peanut, component resolved diagnostics (CRD) was used to better characterize the following groups;

1. Individuals reporting adverse reactions to peanut
2. Peanut skin prick test positives
3. Those with elevated peanut-specific IgE
4. Negative controls

CRD could only be performed for a maximum of 50 subjects due to budgetary limitations.

In the database there were 21 subjects reporting adverse reactions. Of these, 12 subjects had sera samples that were used in the ImmunoCAP analysis. Of the 12 subjects who had sera, only 8 had sufficient volumes of sera (≥350 µL) available for CRD analysis.

Of the 28 subjects in the database who were skin prick test (SPT) positive to peanut, 22 had sera samples that were used in the ImmunoCAP analysis. Of these, 15 had sufficient sera (≥350 µL) left for CRD parameters.

In the database there were 97 subjects with IgE to peanut that was ≥1.5 kU/L. This threshold was chosen to increase the sensitivity for measuring IgE against individual peanut allergens. Of these, 67 had sufficient sera (≥350 µL) and 15 were selected randomly.

For controls, in the database there were 596 subjects with IgE to peanut <0.05 kU/L who reported no symptoms and were peanut SPT negative. Out of these, 489 had sufficient sera and from these, 5 individuals were randomly selected. The total in

the CRD subset was 43 subjects. Figure E3 shows a flowchart of children selected for the CRD component of the study

Supplementary material references

E1. Mills ENC, Mackie AR, Burney P, Beyer K, Frewer L, Madsen C, *et al*. The prevalence, cost and basis of food allergy across Europe. Allergy 2007; 62:717-22.

E2. EuroPrevall: The Prevalence Cost & Basis of Food Allergy. Available from http://www.europrevall.org.

E3. Global View of Food Allergy. Available from http://www.glofal.org.

E4. Final Report Summary - GLOFAL (Global view of food allergy: opportunities to study the influence of microbial exposure) Luxembourg European Commission Community Research and Development Information Service; 2011. Available from http://cordis.europa.eu/.

Figure E1. Map of the Greater Accra region of Ghana and study sites
The map shows the study sites and numbers of enrolled participants at each site.

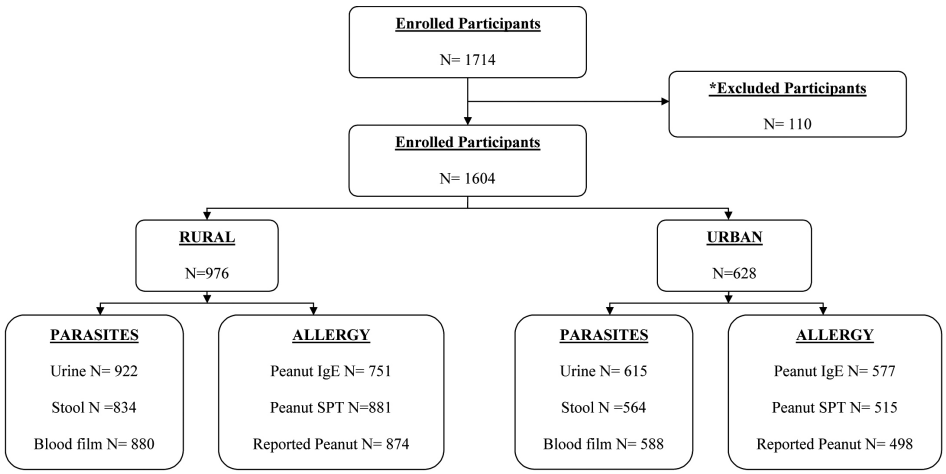

Figure E2: Flowchart of study participants

Flowchart of children recruited from the rural and urban study areas. The number of subjects with complete data for allergy parameters was1004. The number of subjects with complete data for all parameters was 877.

* Excluded participants: 59 enrolled subjects were unavailable for data collection and 51 were outside of the age-range.

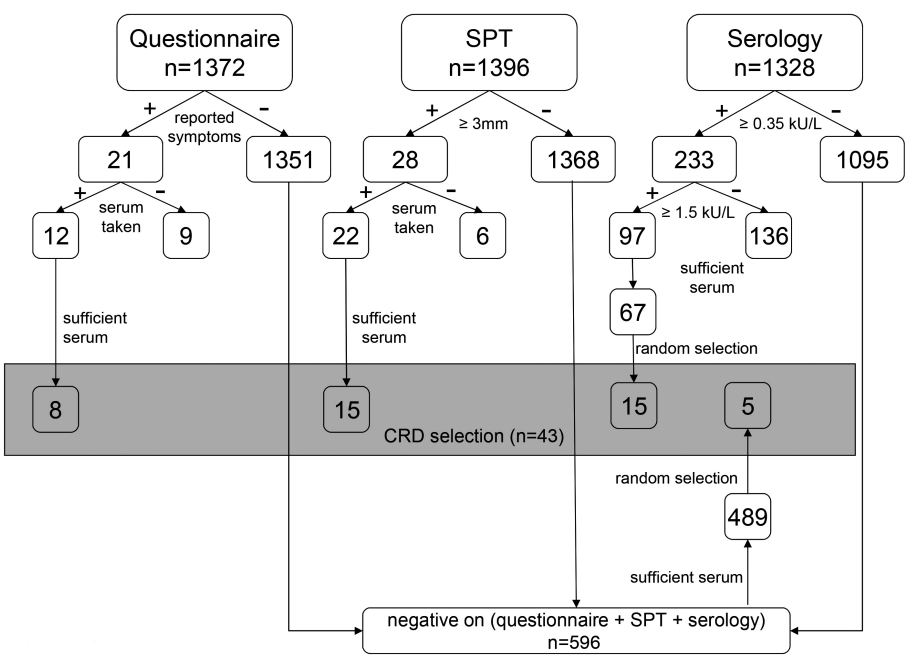

Figure E3. Flowchart of selection for component-resolved diagnostics

Flowchart of children selected for the component-resolved diagnostics component of the study.

Table E1. Characteristics of subset selected for component-resolved diagnostics

ID	Area	Age	Sex	Reported Peanut Reactions	Peanut SPT	Peanut SPT Wheal Size (mm)
AB011	R	7	F	ND	-	0.0
AB041	R	9	M	-	-	0.0
AB051	R	10	F	+	-	0.0
AN052	R	8	F	-	-	0.0
AN068	R	9	M	+	-	0.0
AN084	R	12	F	-	-	0.0
AN102	R	9	F	+	-	0.0
AN115	R	7	M	-	-	0.0
AN132	R	10	M	-	+	3.5
AN142	R	10	M	-	-	0.0
AN143	R	10	F	-	+	3.5
AN190	R	10	M	+	-	0.0
AN218	R	12	M	-	-	0.0
AN220	R	10	F	-	-	0.0
AN228	R	9	M	-	+	4.5
AN248	R	12	M	-	+	5.0
GP001	R	7	M	+	-	0.0
GP031	R	12	F	+	-	0.0
GP074	R	10	M	-	-	0.0
GP094	R	8	M	-	-	0.0
GR063	U	7	M	-	-	1.8
GR098	U	9	M	ND	+	5.0
GR101	U	10	M	-	-	0.0
GR104	U	10	F	-	-	0.0
GR145	U	16	M	-	+	4.0
GR211	U	13	F	-	+	4.0
GR280	U	13	M	-	+	4.5
IP161	U	12	F	-	-	0.0
IP183	U	14	F	ND	+	6.5
IP211	U	15	M	-	+	3.0
IP241	U	6	M	-	-	0.0
IP245	U	13	F	+	-	0.0
KD023	R	10	M	-	-	0.0
KD065	R	11	F	-	-	0.0
KD068	R	10	M	-	-	0.0
KD110	R	12	F	-	-	0.0
KD126	R	12	M	-	-	0.0
KD138	R	12	M	-	+	3.0
NB049	U	7	F	ND	+	3.5
NB071	U	13	M	-	+	3.5
PA058	R	12	M	-	+	3.5
TP095	R	10	M	+	-	0.0
TP116	R	12	F	-	+	3.0

R, rural; ***U***, Urban, ***M***, Male; ***F***, Female; ***+***, Positive; ***-***, Negative; ***SP***, Included in the serum pool for the inhibition assays; **SEA**, *S. haematobium* soluble egg antigen ***BHR***, Included in BHR assays shown; ***ND***, Not done.

ID	Specific IgE (kU/L)							In Serum Pool?	Individual Inhibition by SEA (%)	BHR Assay?
	Peanut	CCD	Ara h 1	Ara h 2	Ara h 3	Ara h 9	Phl p 12			
AB011	24.90	25.90	0.30	0.44	0.48	0.46	0.43	SP	ND	ND
AB041	0.01	0.03	0.03	0.05	0.03	0.09	0.06	ND	ND	ND
AB051	40.20	1.83	0.12	0.18	0.18	72.80	0.29	ND	3.6	BHR
AN052	12.71	10.80	0.15	0.20	0.35	0.26	0.29	SP	ND	ND
AN068	0.00	0.02	0.01	0.02	0.01	0.01	0.03	ND	ND	ND
AN084	12.49	12.50	0.20	0.33	0.26	0.30	0.27	SP	ND	ND
AN102	2.45	2.50	0.11	0.11	0.10	0.25	0.18	ND	ND	ND
AN115	11.17	9.82	0.23	0.39	0.48	0.40	0.44	SP	ND	ND
AN132	0.46	0.44	0.04	0.10	0.06	0.21	0.10	ND	ND	ND
AN142	10.58	17.10	0.14	0.26	0.21	1.80	0.38	SP	ND	ND
AN143	34.59	38.40	0.48	0.80	0.59	0.67	0.58	SP	ND	ND
AN190	0.00	0.02	0.00	0.02	0.01	0.03	0.02	ND	ND	ND
AN218	18.11	25.50	0.19	0.22	0.22	0.33	0.32	SP	ND	ND
AN220	13.82	15.70	0.18	0.35	0.26	0.46	1.08	SP	ND	ND
AN228	2.18	1.79	0.08	0.10	0.07	0.20	0.15	ND	ND	ND
AN248	2.17	2.48	0.11	0.27	0.11	0.27	0.28	ND	ND	ND
GP001	0.03	0.05	0.03	0.06	0.03	0.09	0.09	ND	ND	ND
GP031	10.89	9.59	0.07	0.12	0.12	0.10	0.10	SP	ND	ND
GP074	19.59	16.40	0.27	0.34	0.24	0.34	0.58	SP	ND	ND
GP094	0.04	0.09	0.05	0.06	0.05	0.26	0.09	ND	ND	ND
GR063	13.62	0.77	0.60	1.26	0.64	ND	0.66	ND	ND	ND
GR098	4.51	1.02	0.08	0.09	0.07	0.10	0.13	ND	ND	ND
GR101	5.51	5.02	0.05	0.10	0.07	0.06	0.10	SP	ND	ND
GR104	0.00	0.02	0.01	0.02	0.01	0.00	0.03	ND	ND	ND
GR145	30.02	27.50	0.46	0.69	0.61	11.5	0.53	SP	ND	ND
GR211	21.00	4.89	0.05	0.10	0.06	77.4	0.13	ND	9.2	BHR
GR280	0.99	1.23	0.06	0.08	0.07	0.13	0.08	ND	ND	ND
IP161	1.58	1.27	0.08	0.10	0.07	0.11	0.16	ND	ND	ND
IP183	9.28	8.53	0.31	0.58	0.67	0.61	0.47	SP	ND	ND
IP211	1.20	1.36	0.07	0.12	0.11	0.11	0.12	ND	ND	ND
IP241	0.01	0.47	0.01	0.02	0.01	0.01	0.03	ND	ND	ND
IP245	0.94	0.97	0.14	0.19	0.18	0.18	0.33	ND	ND	ND
KD023	7.81	4.56	0.38	0.44	1.13	0.46	0.71	ND	ND	ND
KD065	29.92	29.70	0.82	1.19	1.01	0.79	1.08	SP	ND	BHR
KD068	20.60	20.40	0.09	0.14	0.11	0.26	0.22	SP	ND	ND
KD110	0.00	0.02	0.01	0.02	0.01	0.00	0.03	ND	ND	ND
KD126	64.28	78.30	0.84	1.22	1.01	0.72	1.04	SP	ND	BHR
KD138	13.14	11.80	0.32	1.13	0.38	0.71	0.42	SP	ND	ND
NB049	2.24	0.10	0.08	0.11	0.07	4.8	0.16	ND	ND	ND
NB071	0.52	0.68	0.09	0.10	0.08	0.15	0.19	ND	ND	ND
PA058	3.72	0.13	0.03	0.04	0.04	9.43	0.09	ND	ND	ND
TP095	0.01	0.03	0.01	0.02	0.02	0.06	0.04	ND	ND	ND
TP116	4.67	2.86	0.06	0.09	0.07	0.06	0.08	ND	83.1	ND

Table E2. Characteristics of subjects reporting adverse reactions to peanut

FACTOR	N (%)
Sex (N=21)	
Male	8 (38.1)
Females	13 (61.9)
Age (N=21)	
<11 years or less	13 (61.9)
>more than 11 years	8 (38.1)
Parasitic Infections	
Any intestinal helminth § positive (N=18)	2 (11.1)
S.haematobium positive (N=18)	1 (5.6)
Plasmodium species* (N=14)	3 (21.4)
Symptoms (N=21)	
Itching, tingling or swelling in the mouth, lips or throat	7 (33.3)
Difficulty swallowing	2 (9.5)
A rash, nettle sting-like rash or itchy skin	0 (0.0)
Diarrhoea or vomiting (other than food poisoning)	14 (66.7)
Runny or stuffy nose	3 (14.3)
Red, sore or running eyes	3 (14.3)
Breathlessness	2 (9.5)
Stiffness in your joints	4 (19.1)
Fainting or dizziness	2 (9.5)
Headaches	7 (33.3)
Reaction Time following Ingestion (N=21)	
Minutes	4 (19.1)
Hours	12 (57.1)
Days	2 (9.5)
Missing Information	3 (14.3)
How Long did Symptoms Last? (N=21)	
Minutes	2 (9.5)
Hours	7 (33.3)
Days	9 (42.9)
Missing Information	3 (14.3)

§ Any intestinal helminth= *Ascaris lumbricoides,* hookworm *(Ancylostoma duodenale* or *Necator americanus)*, *Trichuris trichiura* or *Schistosoma mansoni*.
**Plasmodium* species = *Plasmodium falciparum* or *Plasmodium malariae* (the 2 malaria parasite species detected in our population).

Table E3 Characteristics of subjects reporting adverse reactions with peanut component-resolved diagnostics information

				Reported Peanut Reactions		Peanut	Peanut SPT	Specific IgE (kU/L)						
ID	Area	Age	Sex	Reaction Time	Symptoms	SPT	Wheal Size (mm)	Peanut	CCD	Ara h 1	Ara h 2	Ara h 3	Ara h 9	Phlp12
AB051	R	10	F	Hours	H	-	0.0	40.20	1.83	0.12	0.18	0.18	72.80	0.29
AN068	R	9	M	ND	D	-	0.0	0.00	0.02	0.01	0.02	0.01	0.01	0.03
AN102	R	9	F	Hours	I, F, H	-	0.0	2.45	2.50	0.11	0.11	0.10	0.25	0.18
AN114	R	10	F	Hours	D	-	0.0	ND	ND	ND	ND	ND	ND	ND
AN190	R	10	M	Hours	I, S, D, J, H	-	0.0	0.00	0.02	0.00	0.02	0.01	0.03	0.02
AN193	R	9	M	Hours	D	-	0.0	ND	ND	ND	ND	ND	ND	ND
AN224	R	6	F	Minutes	D	+	5.0	ND	ND	ND	ND	ND	ND	ND
AN227	R	7	M	Days	D	-	0.0	ND	ND	ND	ND	ND	ND	ND
AN242	R	9	F	Days	D	-	0.0	ND	ND	ND	ND	ND	ND	ND
GP001	R	7	M	Hours	D	-	0.0	0.03	0.05	0.03	0.06	0.03	0.09	0.09
GP031	R	12	F	Hours	D	-	0.0	10.9	9.59	0.07	0.12	0.12	0.1	0.10
GR189	U	11	M	Hours	I, N,	-	0.0	0.00	ND	ND	ND	ND	ND	ND
IP239	U	15	F	Minutes	I	ND	ND	ND	ND	ND	ND	ND	ND	ND
IP245	U	13	F	Hours	I, N, E, B, J, H	-	0.0	0.94	0.97	0.14	0.19	0.18	0.18	0.33
KD041	R	10	F	Hours	E, S, B, F, H	-	0.0	ND	ND	ND	ND	ND	ND	ND
KD132	R	11	F	ND	ND	-	0.0	ND	ND	ND	ND	ND	ND	ND
KD167	R	12	M	Minutes	D	-	ND	ND	ND	ND	ND	ND	ND	ND
PA095	R	14	F	Minutes	D	-	0.0	0.07	ND	ND	ND	ND	ND	ND
TP043	R	8	F	Hours	D, F, H	-	0.0	0.26	ND	ND	ND	ND	ND	ND
TP095	R	10	M	ND	I, D	-	0.0	0.01	0.03	0.01	0.02	0.02	0.06	0.04
TP128	R	11	F	Hours	I, S, D, N, E, J, H	-	0.0	0.02	ND	ND	ND	ND	ND	ND

R, rural; ***U***, Urban, ***M***, Male; ***F***, Female; ***+***, Positive; ***-***, Negative; ***ND***, Not done/ No information provided

Reported Symptoms

I, Itching, tingling or swelling in the mouth, lips or throat
D, Diarrhoea or vomiting (other than food poisoning)
B, Breathlessness
S, Difficulty swallowing
N, Runny or stuffy nose
J, Stiffness in your joints
R, A rash, nettle sting-like rash or itchy skin
E, Red, sore or running eyes
F, Fainting or dizziness
H, Headaches

Table E4. Peanut consumption, preparation methods and associations with peanut sensitization (IgE and SPT) and reported adverse reactions to peanut

		Peanut-specific IgE (≥ 0.35 kU/L vs. <0.35 kU/L)		Peanut SPT Positive (+ vs -)		Reported Adverse Reactions to Peanut (Yes vs. No)	
Factors		**Adjusted OR (95%CI)**	**Wald's Test P-value**	**Adjusted OR (95% CI)**	**Wald's Test P-value**	**Adjusted OR (95% CI)**	**Wald's Test P-value**
Peanut Consumption Frequency	Daily (yes vs. no)	1.06 (0.73 – 1.53)	0.76	0.36 (0.09 – 1.40)	0.14	1.06 (0.41 – 2.70)	0.91
	Weekly (yes vs. no)	0.95 (0.68 – 1.32)	0.76	2.81 (0.94 – 8.40)	0.07	0.53 (0.22 – 1.30)	0.17
	Monthly (yes vs. no)	0.91 (0.55 – 1.52)	0.73	0.79 (0.17 – 3.78)	0.77	0.43 (0.06 – 3.32)	0.42
	Never (yes vs. no)	1.07 (0.41 – 2.83)	0.88	----	---	**5.40 (1.47 – 19.80)**	**0.01**
Peanut Preparation Methods	Raw (yes vs. no)	0.74 (0.09 – 5.88)	0.78	5.57 (0.56 – 55.57)	0.14	**17.14 (2.93 – 100.45)**	**0.002**
	Boiling ONLY (yes vs. no)	1.18 (0.55 – 2.54)	0.68	1.14 (0.13 – 9.80)	0.91	----	----
	Frying ONLY (yes vs. no)	0.26 (0.03 – 2.05)	0.20	**17.54 (1.57 – 196.33)**	**0.02**	2.67 (0.34 – 21.29)	0.35
	Roasting ONLY (yes vs. no)	0.90 (0.57 – 1.41)	0.64	2.02 (0.48 – 8.45)	0.34	1.04 (0.37 – 2.82)	0.93
	Peanut oil (yes vs. no)	0.88 (0.27 – 2.85)	0.83	----	----	----	----

Peanut-specific IgE models were adjusted for age, sex, area and *S. haematobium* infection.
Peanut SPT models were adjusted for age, sex, area and peanut-specific IgE.
Reported peanut reaction models adjusted for age, sex and area.
Values in boldface indicate significance.
---- Regression model does not generate estimates.


HAKUNA MATATA
JAPAN

chapter 5

Cellular immune responses and skin prick test reactivity to house dust mite allergen in Ghanaian schoolchildren

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- Manuscript in preparation -

Abstract

Background: In recent years, a marked global rise in the prevalence of allergic disease has been observed. Although a number of factors have been found to account for this increase, few investigations have examined the relationship between cellular immune responses and markers of allergic disease especially in developing countries.

Objective: To study the association between cellular immune responsiveness and house dust mite (HDM) skin prick test (SPT) reactivity among Ghanaian schoolchildren aged 5 to16 years.

Methods: A case-control study was performed in 250 children (95 HDM SPT positive cases 155 controls) selected from a larger cross-sectional investigation. Whole blood samples from study participants were cultured with innate as well as adaptive stimuli for 24 and 72 hours respectively. The innate stimuli used were the TLR 2/1 ligand PAM3CSK4 (Pam3) and the TLR4 ligand lipopolysaccharide (LPS). The adaptive stimuli were purified protein derivative (PPD) and the mitogen phytohaemagglutinin (PHA).

Results: Elevated IL-10 in response to LPS at 24 hours was significantly associated with HDM SPT reactivity (adjusted odds ratio 1.71, 95%CI [1.01 - 2.90], p=0.046). In addition, high IFN-γ to PPD at 72 hours was associated with being a HDM SPT case (adjusted odd ratio 1.77, 95%CI [1.04 - 3.01], p=0.034). No significant associations were observed between cytokine responses to Pam3 or PHA and being a HDM SPT case.

Conclusion: The results of the study suggest that enhanced cellular immune responsiveness to LPS and to PPD are associated with HDM SPT reactivity among Ghanaian children.

Introduction

The prevalence of allergic disorders is on the increase worldwide particularly in low to middle income countries [1] where urbanization and the adoption of a so-called western lifestyle have been linked to the rising incidence of inflammatory diseases [2]. Studies have identified a link between changes in environmental determinants and this increase. Specifically, reduced exposure to pathogens during childhood is thought to be leading to the inadequate maturation of the immune system's regulatory arm thus resulting in uninhibited inflammatory responses toward harmless antigens that include allergens [3, 4]. Key elements of allergic inflammation are mast cells, basophils, eosinophils and immunoglobulin (Ig) E [5]. In addition, during allergic inflammation, T-helper-2 cells regulate type 2 responses through the secretion of cytokines that include IL-4, IL-5, IL-9 and IL-13 [6]. Although type 2 cellular immune responses have been studied extensively in animal models [7] and in a few human studies in western countries [8-10], little is known about these cellular immune responses among allergic children in developing countries.

One commonly used marker of allergic disease in Western countries is atopic sensitization to environmental allergens based on allergen-specific IgE as well as skin prick test (SPT) reactivity. However, a number of studies conducted in helminth-endemic areas have demonstrated the poor diagnostic value of measured allergen-specific IgE due to IgE cross-reactivity [11]. Thus, SPT reactivity to environmental allergens seems more closely related to clinical allergic disease in these countries [12-14] and therefore we have considered this test in our present study.

The aim of our study was to investigate the association between cellular immune responsiveness and house dust mite (HDM) SPT reactivity among schoolchildren living in one region of Ghana.

Materials and methods

Study design and population

We conducted an immunological study among a subset of subjects that had been recruited into a larger cross-sectional study on allergic sensitization and parasitic infections in schoolchildren in Southern Ghana. The cross-sectional survey was conducted between March 2006 and March 2008 and detailed methodology and population description have been reported elsewhere [15]. Briefly, the larger investigation was performed among children aged between 5 and 16 years attending rural and urban schools in the Greater Accra Region. In this cross-sectional study, the percentage of children who were SPT positive to house dust mite allergen was 12.7% (177/1396).

For the current cellular immunological study, an unmatched case-control design was used in which the case definition was house dust mite SPT positivity. Out of 177 HDM SPT positives, 100 randomly selected HDM SPT positives were targeted along with 200 SPT negative controls. Controls were from the same schools as the cases

and were selected in a ratio of one case to two controls. Assessment of immune responsiveness was performed in both groups based on cytokine production following stimulation of whole blood with innate and adaptive stimuli. The study was approved by the Noguchi Memorial Institute for Medical Research Institutional Review Board, Ghana (NMIMR-IRB CPN 012/04-05).

Skin prick testing

For the larger cross-sectional survey, skin prick tests were performed using a panel of food and environmental allergens that included a commercially available extract of house dust mite (*Dermatophagoides* mix - ALK-Abelló, Madrid, Spain). A positive control of histamine hydrochloride (10 mg/ml) and a negative control of saline (ALK-Abelló, Madrid, Spain) were included in the panel. The SPT procedure has been described in detail elsewhere [16]. SPT positivity was defined as a mean wheal diameter ≥3 mm. Controls for the study were SPT negative for all allergens tested.

Whole blood stimulation assay

Four to 6 hours following venipuncture, heparinized blood samples were cultured at a dilution of 1:4 in RPMI 1640 medium (Invitrogen) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin and 2mM glutamate. For the assay, heparinized whole blood was first diluted 1:1 with medium and then 100 µl of the mixture was cultured in each well of a round-bottomed 96-well tissue culture plate (Nunc, VWR International) with 100 µl of either medium alone or stimulus added to medium. The panel of stimuli included innate immune ligands for 24 hour cultures as well as adaptive immune stimuli for 72 hour cultures. The innate stimuli used were the TLR 2/1 ligand PAM3CSK4 (Pam3) and the TLR4 ligand lipopolysaccharide (LPS). The adaptive stimuli were purified protein derivative (PPD) as a marker of adaptive immune response to a vaccine given at birth in Ghana and the mitogen phytohaemagglutinin (PHA) which is a polyclonal T cell stimulus. Both 24 and 72 cultures included responses to culture medium alone as a control. Details of the stimuli used and final concentrations are given in Table 1. A pilot study conducted before the main immunological investigation determined the optimal concentrations to be used. Whole blood cultures were incubated at 37°C in 5% CO_2 for either 24 hours or 72 hours after which supernatants were harvested. Supernatants were frozen at −20°C and later transported on dry ice to a central laboratory for cytokine measurements.

Cytokine measurements

The levels of interleukin IL-10 and tumor necrosis factor (TNF) for 24 hour cultures and IL-10, TNF, interferon (IFN)-γ, IL-13 and IL-17 for 72 hour cultures were determined in harvested supernatants using a Luminex 100 cytometer (Luminex Corporation, Austin Texas, United States) and Luminex cytokine kits (BioSource, Camarillo, California,

Table 1: Panel of stimuli and cytokines measured

STIMULI	ABBR.	CLASSIFICATION	FINAL CONCENTRATION	SOURCE	INCUBATION TIME	CYTOKINES MEASURED
MEDIUM (RPMI)	MED	Negative Control	-	Life technologies	24 hrs. & 72 hrs.	IL-10, TNF (24 hrs.) IL-10, TNF, IFN-γ, IL-13, IL-17 (72 hrs.)
Pam3CSK4	Pam3	TLR2/1 agonist	100 µg /ml	EMC microcollections	24 hrs.	IL-10, TNF
Lipopolysaccharide	LPS	TLR4 agonist	1 ng/ml	Invivogen	24 hrs.	IL-10, TNF
Purified protein derivative	PPD	Tuberculosis antigen	10 µg/ml	Invivogen	72 hrs.	IL-10, TNF, IFN-γ, IL-13
Phytohaemagglutinin	PHA	Mitogen	2 µg/ml	Wellcome Diagnostics, Dartford, UK	72 hrs.	IL-10, TNF, IFN-γ, IL17

United States) according to the manufacturer's instructions. The lower detection limit of the assays was 5 pg/mL for IL-10 and IFN-γ. For TNF, IL-13 and IL-17, the lower detection limit was 10 pg/mL. Samples with concentrations less than the detection limit were assigned one-half the value of this threshold.

Parasitological examinations

For each study participant, one stool sample was collected for the detection of intestinal helminth eggs by the Kato-Katz technique [17] using 25 mg of stool. Intestinal helminths detected were hookworm (*Necator americanus* and *Ancylostoma duodenale*), *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma mansoni*. A urine sample was also collected to determine *Schistosoma haematobium* infection using the standard filtration method [18] in which 10ml of urine is filtered through a nylon nucleopore filter (pore size, 12 μm). A small quantity of blood was used to prepare a Giemsa-stained thick smear slide to detect malaria parasites.

IgE antibody measurements

Immunoglobulin E to house dust mite extract (*Dermatophagoides pteronyssinus*) was assessed using the ImmunoCAP® platform (Thermo Fisher Scientific, Uppsala, Sweden) according to manufacturer's instructions. For our analysis, ≥ 0.35 kU/L was used as the sensitization cut-off.

Anthropometric Measurements

For each study participant, weight and height measurements were determined by weighing scale (BS-8001, capacity: 130 kg) and portable stadiometer respectively. Body Mass Index (BMI) was calculated by dividing weight (kg) by height (m) squared. For each individual, a BMI-for-age z-score based on gender-specific growth chart reference data collected in the World Health Organization Multicentre Growth Reference Study [19] was generated.

Questionnaire

A standard questionnaire was administered to the parents or guardians of study subjects to collect information on demographic and socioeconomic parameters as well as history of asthma within each family. The questionnaire was administered by trained interviewers who were fluent in the local language of each participant. It was pre-tested in a pilot study under field conditions to ensure understanding and acceptability.

Statistical analysis

For our analysis, the exposures of interest were cytokine responses to a panel of innate and adaptive stimuli as assessed by whole blood cultures. Cytokine responses were categorized as either being greater than the median response level (high response)

or below the median response level (low response). Differences between cases and controls according to characteristics of the study population were examined by Pearson's χ^2 tests (with 1 degree of freedom) for categorical variables and Mann-Whitney U test for continuous variables. Crude and adjusted logistic regression models were fitted to determine the associations between immune responses and being a HDM SPT case. In multivariable analysis, models were adjusted for area, age and gender as *a priori* confounders. To examine whether any association between immune response and HDM SPT positivity was different in the urban compared to rural area, for each response, a separate adjusted model was fitted with a product term to examine interaction between immune response and area. A p-value less than 0.05 was taken as the level for statistical significance for multivariable analysis. All analysis was performed using IBM SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of Study Participants

A total of 250 children had complete immunological data and of these, 95 were HDM SPT positive (cases) and 155 SPT negative (controls). Figure 1 shows a flow diagram of study participants from the cross-sectional investigation to the selection for the immunological study. The characteristics of HDM SPT positive cases and controls are presented in Table 2. Overall, a greater proportion of study subjects were from the rural area compared to the urban area as was the case with the larger cross-sectional investigation. In addition, significantly more HDM SPT positives reported wheeze in the last 12 months (p=0.001), doctor-diagnosed asthma (p=0.021) and having an asthmatic father (p=0.038) compared to controls. Specific IgE to house dust mite was also significantly higher among HDM SPT positives compared to controls (p <0.001). With regards to family size, we observed that cases tended to come from families with greater than 6 children while most controls were from families with 1-3 children. However, after adjusting for area, there was no longer an association between family size and being a HDM SPT case. There were no significant differences between cases and controls when it came to parasitic infections or socioeconomic factors.

Innate cytokine responses and HDM SPT positivity

The crude and adjusted associations between cytokine responses to innate stimuli and HDM SPT positivity are shown in Table 3. For IL-10 in response to LPS, a significant association was observed between high response and being an HDM SPT positive case in crude analysis. After adjusting for *a priori* confounders, high IL-10 in response to LPS was still significantly associated with an increased odds of HDM SPT positivity (adjusted odds ratio = 1.71, 95%CI [1.01 - 2.90], p=0.046).

For TNF in response to LPS as well as both cytokine responses to Pam3, high responses were not associated with HDM SPT positivity in crude or multivariable

Table 2: Characteristics of HDM SPT positive cases and controls

FACTORS		Total	HDM SPT +	HDM SPT -	*P-value
Area	Rural	157 (62.8%)	67 (70.5%)	90 (58.1%)	**0.048**
	Urban	93 (37.2%)	28 (29.5%)	65 (41.9%)	
Age	less than 11years	126 (50.4%)	48 (50.5%)	78 (50.3%)	0.975
	11years or greater	124 (49.6%)	47 (49.5%)	77 (49.7%)	
Gender	Male	127 (50.8%)	54 (56.8%)	73 (47.1%)	0.135
	Female	123 (49.2%)	41 (43.2%)	82 (52.9%)	
Family Size	1-3 children	89 (42.8%)	27 (33.8%)	62 (48.4%)	0.075
	4-5 children	48 (23.1%)	19 (23.8%)	29 (22.7%)	
	6+ children	71 (34.1%)	34 (42.5%)	37 (28.9%)	
Birth order	Median (IQR)	2 (1-4)	3 (2-5)	2 (1-4)	0.141
BMI-for-age z-score	Median (IQR)	0.20 (-0.66 – 0.85)	0.12 (-0.64 – 0.85)	0.25 (-0.66 – 0.83)	0.728
Reported Wheeze (12 months)	No	190 (90.9%)	67 (82.7%)	123 (96.1%)	**0.001**
	Yes	19 (9.1%)	14 (17.3%)	5 (3.9%)	
Doctor diagnosed asthma ever	No	195 (93.8%)	72 (88.9%)	123 (96.9%)	**0.021**
	Yes	13 (6.3%)	9 (11.1%)	4 (3.1%)	
Asthma history in child's family	No	118 (57.0%)	42 (52.5%)	76 (59.8%)	0.114
	Yes	72 (34.8%)	34 (42.5%)	38 (29.9%)	
	No Idea	17 (8.2%)	4 (5.0%)	13 (10.2%)	
Asthmatic mother	No	196 (93.8%)	78 (96.3%)	118 (92.2%)	0.231
	Yes	13 (6.2%)	3 (3.7%)	10 (7.8%)	
Asthmatic father	No	199 (95.2%)	74 (91.4%)	125 (97.7%)	**0.038**
	Yes	10 (4.8%)	7 (8.6%)	3 (2.3%)	
Specific IgE to House Dust mite	< 0.35 kU/L	143 (63.3%)	21 (25.9%)	122 (84.1%)	**<0.001**
	≥ 0.35 kU/L	83 (36.7%)	60 (74.1%)	23 (15.9%)	
**Any Helminth	Negative	179 (72.8%)	70 (76.1%)	109 (70.8%)	0.366
	Positive	67 (27.2%)	22 (23.9%)	45 (29.2%)	
***Plasmodium species	Negative	176(72.1%)	64(69.6%)	112(73.7%)	0.487
	Positive	68(27.9%)	28(30.4%)	40(26.3%)	
Primary household fuel	Firewood	75 (36.1%)	34 (42.0%)	41 (32.3%)	0.349
	Charcoal /firewood/ Kerosene	74 (35.6%)	27 (33.3%)	47 (37.0%)	
	LPG/Electricity	59 (28.4%)	20 (24.7%)	39 (30.7%)	
Main toilet option	No toilet	95 (45.7%)	39 (48.1%)	56 (44.1%)	0.189
	Public toilet	33 (15.9%)	17 (21.0%)	16 (12.6%)	
	Shared toilet	46 (22.1%)	13 (16.0%)	33 (26.0%)	
	Indoor /private toilet	34 (16.3%)	12 (14.8%)	22 (17.3%)	
Main water source	River/ Untreated water	100 (47.8%)	44 (54.3%)	56 (43.8%)	0.281
	Well/Borehole	43 (20.6%)	16 (19.8%)	27 (21.1%)	
	Piped /Treated water	66 (31.6%)	21 (25.9%)	45 (35.2%)	

* χ^2 Test or Mann-Whitney U Test p-value.
**Any Helminth = *Schistosoma haematobium, Schistosoma mansoni*, hookworm hookworm (*Ancylostoma duodenale* or *Necator americanus*), *Ascaris lumbricoides or Trichuris trichiura.*
****Plasmodium* species = *Plasmodium falciparum or Plasmodium malariae (the 2 malaria species detected in our population.*
P-values less than 0.05 are shown in bold.

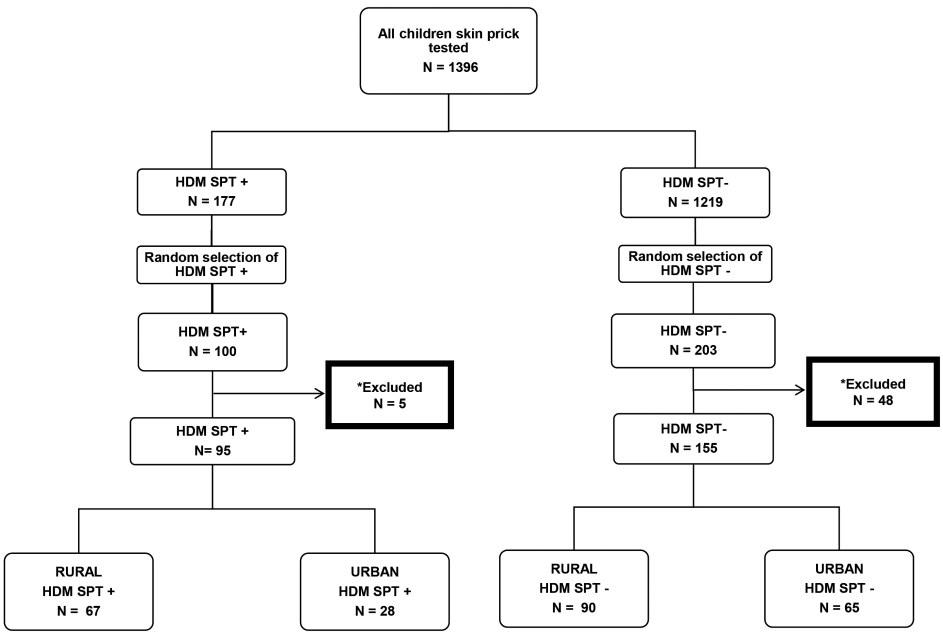

Figure 1: Flowchart of study participants
Flowchart of children selected for the immunological study.
* Subjects were excluded for missing immunological data.

analyses as shown in Table 3. The levels of IL-10 and TNF in response to medium at 24 hours were low and were not related to being a HDM SPT case in crude or multivariable analyses.

The results of adjusted models fitted with product terms to explore interaction between immune response and area are also shown in Table 3. The p-values for the interaction terms ranged from p=0.321 to p=1.000 indicating no evidence that the associations between immune response and HDM SPT reactivity was different in the urban and rural areas for the 24 hour cultures.

Adaptive cytokine responses and HDM SPT positivity

Tables 4A and 4B show the results of the crude and multivariable analyses of HDM SPT positivity and cytokine responses to stimuli that are considered to stimulate the adaptive immune system. For PPD, greater proportions of HDM SPT cases had high responses (i.e. above the median level) for IL-10, TNF and IFN-γ compared to SPT negative controls. In crude logistic regression analysis, high IFN-γ in response to PPD was significantly associated with HDM SPT positivity. This remained significant after adjusting for *a priori* confounders (adjusted OR 1.77 95%CI [1.04 - 3.01], p=0.034). For IL-10 in response to PPD, although the measure of effect from the crude logistic

Table 3: Crude and adjusted associations between immune responses and HDM SPT positivity (24 hour responses)

Stimuli	Cytokine	TOTAL N (%)	HDM SPT + N (%)	HDM SPT - N (%)	Crude Odds Ratio (95% CI)	Wald's Test P-value	Adjusted Odds Ratio (95% CI)	Wald's Test P-value	p-value Interaction Area * Immune Response
MEDIUM	IL-10 ≤2.5 pg/ml	224 (89.6%)	87 (91.6%)	137 (88.4%)	1.00	-	1.00	-	1.000
	IL-10 >2.5 pg/ml	26 (10.4%)	8 (8.4%)	18 (11.6%)	0.70 (0.29 - 1.68)	0.424	0.59 (0.24 - 1.45)	0.247	
	TNF ≤5.0 pg/ml	212 (84.8%)	80 (84.2%)	132 (85.2%)	1.00	-	1.00	-	0.820
	TNF >5.0 pg/ml	38 (15.2%)	15 (15.8%)	23 (14.8%)	1.08 (0.53 - 2.18)	0.839	0.94 (0.45 - 1.95)	0.865	
PAM	IL-10 ≤140.4 pg/ml	125 (50.0%)	44 (46.3%)	81 (52.3%)	1.00	-	1.00	-	0.323
	IL-10 >140.4 pg/ml	125 (50.0%)	51 (53.7%)	74 (47.7%)	1.27 (0.76 - 2.12)	0.362	1.19 (0.70 - 2.02)	0.511	
	TNF ≤267.5 pg/ml	125 (50.0%)	46 (48.4%)	79 (51.0%)	1.00	-	1.00	-	0.696
	TNF >267.5 pg/ml	125 (50.0%)	49 (51.6%)	76 (49.0%)	1.11 (0.66 - 1.85)	0.696	1.03 (0.61 - 1.74)	0.905	
LPS	IL-10 ≤151.6 pg/ml	126 (50.4%)	40 (42.1%)	86 (55.5%)	1.00	-	1.00	-	0.551
	IL-10 >151.6 pg/ml	124 (49.6%)	55 (57.9%)	69 (44.5%)	**1.71 (1.02 - 2.87)**	**0.041**	**1.71 (1.01 - 2.90)**	**0.046**	
	TNF ≤568.2 pg/ml	125 (50.0%)	46 (48.4%)	79 (51.0%)	1.00	-	1.00	-	0.321
	TNF >568.2 pg/ml	125 (50.0%)	49 (51.6%)	76 (49.0%)	1.11 (0.66 - 1.85)	0.696	1.10 (0.66 - 1.85)	0.719	

Models adjusted for age, gender and area.
P-values less than 0.05 are shown in bold.

regression model showed that this response was linked to 1.5 times the odds of a HDM SPT positive case, this was only of borderline significance and in the adjusted model, no longer significant (p=0.132).

Although TNF in response to PPD was not significantly associated with HDM SPT positivity in either crude or multivariable analyses, there was evidence of significant interaction between area and this immune response. Therefore, new models were fitted in which the association between TNF in response to PPD and HDM SPT positivity was examined after stratifying for area. In the rural area, high TNF in response to PPD was associated with HDM SPT positivity after adjusting for confounders (adjusted OR =1.98 95%CI [1.02 – 3.83], p=0.043); this was not seen in the urban area where very fewer cases had elevated TNF in response to PPD compared to controls. For PHA, all cytokine responses were high. However, there were no significant associations between cytokine responses and being an HDM SPT case as shown in Table 4B. From the 72 hour cultures, cytokine responses to medium were generally all low. In addition, no significant associations were observed between cytokine responses to medium and HDM SPT positivity (shown in Table 4A).

Table 4A and 4B also show the results of models fitted with product terms to examine evidence of interaction between cytokine responses and area. For IL-13 and IL-17, the p-values for the interaction term were p> 0.700. For IL-10, TNF, IFN-γ and IL-13 in response to PPD, the significance of the interaction terms ranged from p=0.386 to p=0.845 while for cytokine responses to PHA, the range was from p=0.153 to p=0.915. Therefore, for these specific responses, there was no evidence of significant interaction between area and immune response.

Discussion

We investigated associations between immune responsiveness and allergic sensitization based on house dust mite SPT positivity among urban and rural children in Ghana. A number of factors previously reported to be linked to allergic sensitization based on SPT reactivity were significantly associated with HDM SPT positivity in our study such as wheeze [20], doctor-diagnosed asthma [21], family history of allergic diseases [22] and elevated specific IgE to dust mite [23]. Therefore, HDM SPT positivity was indeed an appropriate marker for the pathogenesis of allergy in our population. Although factors associated with allergic sensitization have been explored in similar settings [20, 24], few studies have examined the effect of cellular immune responsiveness on allergy outcomes.

With regards to innate cytokines, elevated LPS-induced IL-10 was significantly associated with HDM SPT positivity in our study. LPS is a key component of the outer membrane of Gram-negative bacteria that can initiate strong innate immune responses in humans [25]. Our findings seem somewhat unexpected since there are data showing that IL-10 is a suppressory cytokine [26]. Furthermore, a small study conducted among European allergic asthmatic children observed that IL-10 production in whole blood stimulated with LPS was

Table 4: Crude and adjusted associations between immune responses and HDM SPT positivity (72 hour responses)
A. Medium

Stimuli	Cytokine	TOTAL N (%)	HDM SPT + N (%)	HDM SPT - N (%)	Crude Odds Ratio (95% CI)	Wald's Test P-value	Adjusted Odds Ratio (95% CI)	Wald's Test P-value	Area * Immune Response p-value for interaction
MEDIUM	IL-10 ≤2.5 pg/ml	239 (95.6%)	89 (93.7%)	150 (96.8%)	1.00	-	1.00	-	***
	IL-10 >2.5 pg/ml	11 (4.4%)	6 (6.3%)	5 (3.2%)	2.02 (0.60 - 6.82)	0.256	1.87 (0.54 - 6.51)	0.327	
	TNF ≤5.0 pg/ml	230 (92.0%)	89 (93.7%)	141 (91.0%)	1.00	-	1.00	-	****
	TNF >5.0 pg/ml	20 (8.0%)	6 (6.3%)	14 (9.0%)	0.68 (0.25 - 1.83)	0.445	0.54 (0.19 - 1.49)	0.233	
	IFN-γ ≤2.5 pg/ml	245 (98.0%)	93 (97.9%)	152 (98.1%)	1.00	-	1.00	-	****
	IFN-γ >2.5 pg/ml	5 (2.0%)	2 (2.1%)	3 (1.9%)	1.09 (0.18 - 6.64)	0.926	0.83 (0.13 - 5.18)	0.841	
	IL-13 ≤5.0 pg/ml	180 (72.0%)	69 (72.6%)	111 (71.6%)	1.00	-	1.00	-	0.723
	IL-13 >5.0 pg/ml	70 (28.0%)	26 (27.4%)	44 (28.4%)	0.95 (0.54 - 1.68)	0.862	0.95 (0.53 - 1.70)	0.864	
	IL-17 ≤5.0 pg/ml	173 (69.2%)	64 (67.4%)	109 (70.3%)	1.00	-	1.00	-	0.703
	IL-17 >5.0 pg/ml	77 (30.8%)	31 (32.6%)	46 (29.7%)	1.15 (0.66 - 1.99)	0.623	0.93 (0.52 - 1.66)	0.802	

Models adjusted for age, gender and area.
P-values less than 0.05 are shown in bold.
**** Interaction term dropped from model since no urban subjects had cytokine levels greater than the median for this immune response.

B. PPD and PHA

Stimuli	Cytokine	TOTAL N (%)	HDM SPT + N (%)	HDM SPT - N (%)	Crude Odds Ratio (95% CI)	Wald's Test P-value	Adjusted Odds Ratio (95% CI)	Wald's Test P-value	Area * Immune Response p-value Interaction
PPD	IL-10 ≤10.3 pg/ml	125 (50.0%)	41 (43.2%)	84 (54.2%)	1.00	-	1.00	-	0.386
	IL-10 >10.3 pg/ml	125 (50.0%)	54 (56.8%)	71 (45.8%)	1.56 (0.93 - 2.61)	0.091	1.50 (0.89 - 2.54)	0.132	
	TNF ≤5.0 pg/ml	153 (61.2%)	53 (55.8%)	100 (64.5%)	1.00	-	1.00		**0.028**
	TNF >5.0 pg/ml	97 (38.8%)	42 (44.2%)	55 (35.5%)	1.44 (0.86 - 2.43)	0.170	1.31 (0.75 - 2.31)	0.342	
	IFN-γ ≤15.7pg/ml	125 (50.0%)	38 (40.0%)	87 (56.1%)	1.00	-	1.00	-	0.493
	IFN-γ >15.7pg/ml	125 (50.0%)	57 (60.0%)	68 (43.9%)	**1.92 (1.14 - 3.22)**	**0.014**	**1.77 (1.04 - 3.01)**	**0.034**	
	IL-13 ≤5.0 pg/ml	136 (54.4%)	51 (53.7%)	85 (54.8%)	1.00	-	1.00		0.845
	IL-13 >5.0 pg/ml	114 (45.6%)	44 (46.3%)	70 (45.2%)	1.05 (0.63 - 1.75)	0.859	0.99 (0.58 - 1.69)	0.956	
PHA	IL-10 ≤59.4 pg/ml	125 (50.0%)	49 (51.6%)	76 (49.0%)	1.00		1.00	-	0.915
	IL-10 >59.4 pg/ml	125 (50.0%)	46 (48.4%)	79 (51.0%)	0.90 (0.54 - 1.51)	0.696	0.78 (0.46 - 1.32)	0.356	
	TNF ≤ 8.6 pg/ml	126 (50.4%)	45 (47.4%)	81 (52.3%)	1.00		1.00	-	0.153
	TNF > 8.6 pg/ml	124 (49.6%)	50 (52.6%)	74 (47.7%)	1.22 (0.73 - 2.03)	0.453	1.08 (0.63 - 1.84)	0.788	
	IFN-γ ≤23.4 pg/ml	125 (50.0%)	46 (48.4%)	79 (51.0%)	1.00		1.00	-	0.493
	IFN-γ >23.4 pg/ml	125 (50.0%)	49 (51.6%)	76 (49.0%)	1.11 (0.66 - 1.85)	0.696	1.05 (0.62 - 1.77)	0.856	
	IL-17 ≤50.0 pg/ml	125 (50.0%)	50 (52.6%)	75 (48.4%)	1.00		1.00	-	0.803
	IL-17 >50.0 pg/ml	125 (50.0%)	45 (47.4%)	80 (51.6%)	0.84 (0.51 - 1.41)	0.515	0.78 (0.46 - 1.31)	0.342	

Models adjusted for age, gender and area.
P-values less than 0.05 are shown in bold.

significantly lower in the allergic group compared to healthy controls [27]. Although IL-10 will be discussed later, it is important to note that responses to innate immune ligands have been shown to vary greatly across different populations worldwide. For example, an investigation of innate immune responses to TLR ligands measured among infants across four continents (Africa, Europe, North America and South America) using standardized methodology observed that South African infants had lower responsiveness to TLR ligands including LPS compared to infants from the three other sites [28].

An investigation from a helminth-endemic area of Gabon, Central Africa reported that children infected with *S. haematobium* showed lower immune responsiveness when their peripheral blood mononuclear cells were stimulated with LPS compared to uninfected children [29]. It is also known that repeated exposure to TLR ligands such as LPS can lead to a dampening of response in what is known as TLR tolerance [30]. Therefore, it is possible that both the infected children in the study from Gabon and the controls in our study had greater exposure to bacteria and were tolerant to LPS and thus less responsive in general. However, additional studies would be needed to investigate this in our population especially since significant observations were made for LPS only but not for the TLR2/1 ligand Pam3.

PPD, which is a mixture of mycobacterial antigens, was used because 94.8% of our study participants with verifiable records had received BCG vaccine at birth. The magnitude of IFN-γ production in response to PPD by whole blood assay is widely used to assess immunological protection against tuberculosis following BCG vaccination [31]. As this response can be measured years after vaccination [32], PPD provides a suitable antigen to assess adaptive immune responses. With regards to this adaptive immune responsiveness, high IFN-γ in response to PPD was significantly associated with HDM SPT positivity in our study. Moreover, in the rural area, high TNF in response to PPD was significantly associated with being HDM SPT positive.

Although no longer significant after multivariable analysis, IL-10 to PPD was also associated with SPT to HDM. The higher IFN-γ and IL-10 adaptive responses in allergic subjects were not expected. Earlier studies not only in Gabon [33] and Vietnam [34] but also in Australia [35] have shown that the immune suppressory cytokine, IL-10, is negatively associated with SPT. Moreover, in terms of the balance between Th1 and Th2, allergic subjects would be expected to have lower IFN-γ responses [36]. These findings along with the high IL-10 to LPS being associated with SPT positivity, suggest that greater immune responsiveness, rather than a specific cytokine, is associated with HDM positivity.

Similar observations were made among urban children in Brazil where cytokine responses from whole blood cultures stimulated with a mitogen were measured in 1127 children [37]. In this study, the responsive immune phenotype was characterized by generalized production of cytokines above limits of detection [37]. The cytokines measured in the Brazilian study were IFN-γ, IL-5, IL-13 and IL-10, and findings from this investigation indicate that general enhanced responsiveness based on these cytokines was associated with increased odds of SPT reactivity as well as allergen-specific IgE sensitization [37].

Although we observed enhanced cytokine production linked to HDM SPT positivity with PPD, no significant associations were seen in our study when responses to the mitogen PHA and SPT reactivity were considered, which is in contrast to the Brazilian study. However, there were some notable differences between our study and the investigation from Brazil in that they used the mitogen pokeweed which is not as strong a stimulus and assessed cytokines after 120 hours of stimulation instead of 72 hours in our case [37]. Different population dynamics and study methodologies make direct comparisons of cellular immune findings between investigations very problematic.

Our study had a number of limitations such as the relatively small sample size which meant reduced statistical power for some of the associations examined. This reduced power is reflected in some of the borderline significant associations observed and we cannot therefore rule out the possibility of type 2 errors in our study. Moreover, additional studies are needed that measure not only cytokines in supernatants but also at a single cell level in order to identify which cells contribute to the cytokine network in allergic individuals compared to non-allergics.

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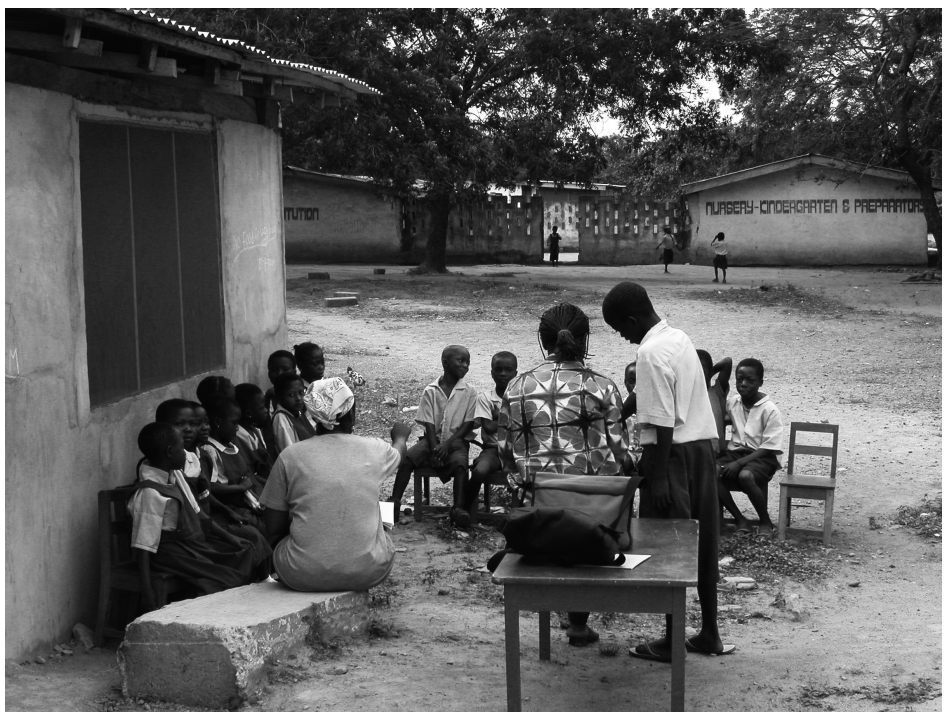
NURSERY-KINDERGARTEN & PREPARATORY

chapter 6

Urban-rural differences in the gene expression profiles of Ghanaian children

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Abstract

Recent studies indicate that urbanization is having a pronounced effect on disease patterns in developing countries. To understand the immunological basis of this, we examined mRNA expression in whole blood of genes involved in immune activation and regulation in 151 children aged 5-13 years attending rural, urban low socioeconomic status (SES) and urban high SES schools in Ghana. Samples were also collected to detect helminth and malaria infections.

Marked differences in gene expression were observed between the rural and urban areas as well as within the urban area. The expression of both interleukin (IL)-10 and programmed cell death protein 1 (PD-1) increased significantly across the schools from urban high SES to urban low SES to rural (p-trend<0.001). Although IL-10 gene expression was significantly elevated in the rural compared to the urban schools (p <0.001), this was not associated with parasitic infection. Significant differences in the expression of Toll-like receptors (TLRs) and their signalling genes were seen between the two urban schools. Genetic differences could not fully account for the gene expression profiles in the different groups as shown by analysis of *IL-10*, *TLR-2* and *TLR-4* gene polymorphisms.

Immune gene expression patterns are strongly influenced by environmental determinants and may underlie the effects of urbanization seen on health outcomes.

Key words

Gene expression, polymorphisms, urbanization, Interleukin-10, Toll-like Receptors, helminths,

Introduction

Urbanization worldwide, particularly in developing nations, is changing mortality and morbidity patterns from largely infectious to non-communicable diseases [1]. Although governed by genetics, the increase in non-communicable (often inflammatory) diseases is thought to be driven by changes in environmental factors [2]. Moreover, in rapidly expanding urban centres, socioeconomic differences are resulting in heterogeneous environmental exposures that are determining disease patterns. For instance, some urban areas are characterized by high population density, overcrowding, limited access to potable water and poor sanitation [3], all of which increase exposure to pathogens [4]. Conversely, other urban environments are more affluent with wealthier inhabitants having greater access to clean water, food and adequate sanitation but at the same time being more susceptible to chronic conditions such as hypertension [5], obesity [6] and cardiovascular diseases [7]. Further along this spectrum, rural environments in developing countries remain largely agrarian with lifestyles characterized by traditional diet [8], limited access to health-care [9] and in many areas, continual exposure to pathogens.

Understanding the factors associated with changing environments and the link to the alteration of the immune system would be important for both communicable and non-communicable disease prevention strategies. Although risk factors associated with the rural to urban transition, particularly in relation to inflammatory diseases, have been studied extensively [10,11], little is known about actual changes that take place in the immune system as a function of the rural-urban gradient.

Examining gene expression patterns is one approach towards dissecting differences in immune responses between urban and rural populations since variability in gene expression is a result of not only genetic but also environmental factors [12]. Moreover, differential gene expression can be a key mechanism in disease manifestation [13]. Although many studies have addressed the genetic determinants of gene expression, few have examined the impact of geographical location in generating transcriptional variation. A study conducted among the genetically homogenous Amazighs of Morocco living in three geographically distinct areas demonstrated that locality can have a dominant impact on gene expression profiles with up to one third of the leukocyte transcriptome being associated with geographical area differences [14, 15]. However, this study did not explore the possible factors within the distinct geographical locations that may account for observed profile differences. The high burden of parasitic infection in many rural areas, in addition to differences in socioeconomic status (SES) within urban areas, can result in very different exposures to microorganisms and thus lead to differences in transcriptomal profiles.

We investigated whether contrasting geographical locations in one region of Ghana with a large urban centre and rural areas that are endemic for parasitic infections [16] have an impact on messenger RNA (mRNA) expression of selected immune genes among children. Our target study locations were a rural area, an urban low SES area and an urban high SES area.

Of particular interest were genes involved in immune activation and regulation particularly in response to parasites including helminths. Given the role of Toll-like receptors (TLRs) in the recognition of pathogen-associated molecular patterns (PAMPs) linked to microbial infection [17], various genes involved in the TLR signalling pathway were part of our selection. In environments typified by chronic helminth infections, key factors involved in the immune regulation of helminth infections such as the regulatory cytokines IL-10 and TGF-β [18] were part of our selection. In addition, immune markers involved in T-cell activation and polarization such as FOXP3 were included since CD4+CD25+FOXP3+ regulatory cells form a key population of regulatory cells involved in infections in general [19]. The gene for immunoglobulin E (IgE) antibody, which is strongly associated with immune responses to helminths, was included to compare with IgE antibody levels in circulation and thus act as a control. We hypothesized that the expression of genes involved in immune regulatory processes associated with helminth infections would be high in the rural area followed by the urban low SES and then urban high SES areas.

Methods

Study area

Study participants were recruited from 3 schools located in distinct geographical locations of the Greater Accra region of Ghana. This region is the second most populous in Ghana and is situated in the south-east of the country. The 3 schools were selected to reflect the dynamic environmental changes associated with urbanization in Ghana.

The rural school was located in Ayikai Doblo, a community in the Ga West district which is approximately 20-30 km north of Accra City Centre. The vast majority of people in Ayikai Doblo are of the Ga-Adangme ethnic group. The main income earning activities in this community are farming, trading and commercial sand collection. The area remains endemic for the waterborne helminth infection *S. haematobium* [16]. The urban low SES school was located in Jamestown a coastal community in the city of Accra and is inhabited predominantly by indigenous Ga-Adangme people. Jamestown can best be described as a "large high-density low-income formal settlement" [20] and is characterized by overcrowding as well as poor sanitation. The main economic activities in this area revolve around fishing and petty trading. The urban high SES school was situated on the University of Ghana campus at Legon and can be classified as middle-to-high income with the majority of those attending this school being the children of faculty and employees of the university. The school is ethnically diverse with not only Ga-Adangmes but also other Ghanaian ethnic groups.

Study Population

The study population consisted of schoolchildren aged between 5 - 13 years randomly selected from a larger investigation into immune responses, parasitic infections and atopic sensitization in Ghanaian children [21].

RNA isolation from whole blood

For each study subject, blood was drawn into a heparinized tube and immediately following venipuncture, 0.8 ml of whole blood was added to 3.6 ml of Nuclisens lysis buffer (Biomérieux, Boxtel, The Netherlands) to stabilize RNA. Samples were stored for a maximum of 2 weeks at 4°C after which they were transferred to -80 °C for long-term storage. Detailed RNA isolation methodology has been described previously [21]. Briefly, a Nuclisens isolation kit (Biomérieux, Boxtel, The Netherlands) was used for the isolation of total nucleic acid according to manufacturer's instructions. Prior to the isolation, the samples were treated with RNAse-free DNAse (Invitrogen, Breda, The Netherlands) to remove genomic DNA.

cDNA synthesis and Real-time PCR

The cDNA synthesis and real-time PCR methodology followed has been described in detail by Hartgers *et al.* [21] Briefly, reverse transcription of RNA was carried out using moloney murine leukaemia virus reverse transcriptase (Invitrogen, Breda, The Netherlands). Gene expression was determined by real-time quantitative PCR using the ABI PRISM 7500 system (Applied Biosystems, Foster City, California, USA). PCR reactions were performed in duplicate according to Taqman™ assay instructions using Taqman probes and qPCR Core kit reagents (Eurogentec, Seraing, Belgium).

Normalization of gene expression was done using the housekeeping gene 18S rRNA. Following the normalization procedure, the donor with the lowest expression was set to 1. Expression levels for other donors for each gene were determined relative to this donor. A description of genes examined is shown in Table S1 of the supplementary material.

Genotyping

Polymorphisms of *IL-10, TLR-2* and *TLR-4* genes were selected on the basis of a larger investigation to establish whether genetic variants associated with allergic phenotypes in developed countries were of relevance in Ghana. Therefore, variants associated with allergy phenotypes in European populations were targeted.

For IL-10, tagging SNPs from the IL-10 gene region were selected based on genotype data available through the HapMap project (www.hapmap.org). The HapMap reference population for the tagging selection was the CEPH (Utah Residents with Northern and Western European Ancestry). In addition, SNPs in close linkage disequilibrium with the tagging SNPs as well as an additional IL-10 promoter SNP of functional importance in IL-10 cytokine production (rs10494879) were included. The linkage disequilibrium plot for the IL-10 SNPs genotyped is shown in Figure S5 (Supplementary material).

TLR-2 and *TLR-4* polymorphisms were selected from the 10 human *TLR* genes using the Innate Immunity Program for Genomic Applications mutation screen [22]. Polymorphisms with a minor allele frequency ≥ 0.03 associated with amino acid

changes as well as SNPs with a minor allele frequency > 0.1 associated with altered transcription factor binding in *TLR* regulatory regions were selected [22].

All SNPS were genotyped by matrix-assisted laser desorption / ionization time-of-flight mass spectrometry using the MassARRAY system (Sequenom Inc, San Diego, California, USA) as has been described in detail elsewhere [22, 23]. A total of 330 samples from our study population were genotyped successfully for the IL-10 gene and 318 for the *TLR-2* and *TLR-4* genes. Genotype frequencies in the study population were examined for deviation from Hardy-Weinberg Equilibrium (HWE) as part of quality control for the genotyping process. MAFs for the SNPs genotyped were also compared to Yoruba in Ibadan, Nigeria genotype data from HapMap (www.hapmap.org) as well as to studies on genetic variation and inflammatory responses conducted in Northern Ghana [24, 25] (see Table S3 and S6 - Supplementary material).

Total IgE and CRP

Serum levels of total IgE as well as CRP were assessed for each participant. Total IgE was measured by enzyme linked immunosorbent assay as described in detail elsewhere [26]. The concentration of CRP in serum samples was determined by immunoturbidimetric assay using the automated P-800 system (Hitachi, Tokyo, Japan). Detailed methodology has been described elsewhere [27].

Detection of parasitic infections

Helminths

Stool samples were collected for the detection of intestinal helminth eggs by the Kato-Katz technique using 25 mg of stool. A urine sample was also collected to determine *S. haematobium* infection using the standard filtration method in which 10ml of urine is filtered through a nylon nucleopore filter (pore size, 10 µm) in a Swin-lok filtration device (Whatman,'s-Hertogenbosch, Netherlands). Helminth eggs were detected by microscopy.

Malaria detection

For each subject, a small quantity of blood was used to prepare a Giemsa-stained thick smear slide to detect malaria parasites by microscopy.

Statistical analyses

Area differences in the distribution of subject characteristics were examined by Pearson's χ^2 tests for categorical variables and Mann-Whitney U test for continuous variables. A p-value less than 0.05 was taken as the level for statistical significance. Messenger RNA expression levels were not normally distributed and so were log-transformed (base 10). Z-scores [(individual level – mean level) / standard deviation] were generated on log-transformed mRNA data. Analysis of Covariance (ANCOVA) models were used to examine the association between area and mRNA expression levels adjusting for age

and gender as *a priori* confounders. The Bonferroni correction was applied for between area pair-wise comparisons. We also used ANCOVA models to examine variations in mRNA expression according to helminth infections and malaria infection.

Haploview software package [28] was used to estimate minor allele frequencies of *IL-10, TLR-2* and *TLR-4* gene polymorphisms. Deviations from HWE were tested by χ^2 tests. Area differences in the SNP MAFs were also examined. Linear regression models were generated to examine the relationship between individual *IL-10* SNPs and IL-10 mRNA expression correcting for age, gender and area assuming an additive model. The same was done for the effects of *TLR-2* and *TLR-4* SNPs on TLR-2 and TLR-4 mRNA expression levels respectively.

Statistical analysis was performed using IBM SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA) was used to generate figures.

Informed consent and ethical approval

The parents / guardians of all study participants were given informed consent forms to sign or thumbprint if they wished to enrol their wards in the study. They were also provided with study information sheets which were explained verbally at Parent Teacher Association (PTA) meetings held at the urban low SES and rural schools. Ethical approval for this project was granted by the Noguchi Memorial Institute for Medical Research Institutional Review Board (approval number CPN015/02-03).

Results

Characteristics of study participants

Whole blood samples were collected from 151 children for gene expression profiling. Table 1 shows the characteristics of the study participants. The rural area was endemic for helminths with 54.2% of children being positive for *Schistosoma haematobium* and 38.3% having at least one intestinal helminth infection. Malaria infection was detected among 52.2% of rural participants. Both urban schools were free of *S. haematobium* infection with no detectable malaria infection among the urban low SES children and one case among urban high SES children. Intestinal helminth infections were present in both urban schools and affected 13.5% of urban low SES participants compared to 2.0% of urban high SES subjects. Serological analysis showed that the geometric mean c-reactive protein (CRP) level was 5 times higher among rural children compared to their urban counterparts and slightly lower in the urban low SES compared to urban high SES school. We also observed that the geometric mean total IgE level was significantly elevated among rural compared to both urban low SES and urban high SES children ($p < 0.001$).

Living in the rural area was associated with elevated total IgE (but not CRP) after taking parasitic infections into account

We analyzed whether area differences in CRP and total IgE levels still remained after taking parasitic infections into account. Given that these parameters were not normally distributed, CRP was categorized into a binary variable using the geometric mean (1.1 mg/ml) as a cut-off while total IgE was log-transformed. After adjusting for current helminth and malaria infections, living in the rural area still was strongly associated with elevated total IgE ($p < 0.001$) but not CRP ($p=0.232$). In addition, malaria infection was independently associated with CRP ($p < 0.05$) after adjusting for area.

Gene expression profiles can vary as a function of urban-rural area & SES

Gene expression levels (expressed as z-scores) in peripheral blood samples of children in the three areas were compared and are shown in Figure 1.The results of detailed Bonferroni pairwise comparisons of between-area differences in gene expression levels are shown in Table S2 (Supplementary material). IgE mRNA expression was strongly elevated among children in the rural area, where helminths were highly prevalent, compared to both urban schools. A correlation was observed between total IgE and IgE mRNA (Spearman's rho correlation coefficient = 0.61, $p < 0.001$). The expression levels for genes with immunosuppressive activities such as IL-10 and PD-1 were highest among rural participants followed by the urban low SES school and lastly the urban high SES school (p-trend < 0.001). Messenger RNA levels for IL-10 and for PD-1 were also correlated with each other (Spearman's rho correlation coefficient = 0.55, $p < 0.001$) as shown in Figure S1 (Supplementary material). Other genes involved in immune regulation such as TGF-β and FOXP3 were lowest in the rural area and while TGF-β levels showed a gradient increase across the schools from the rural to urban low SES to urban high SES, FOXP3 expression was similar in both urban schools.

The expression of some genes involved in TLR signalling, specifically TLR-2, TLR-4, CD14, NOD-2, SOCS-3 and LIR-7 were all high in the blood of urban high SES children but lower in both urban low SES and rural children. Post-hoc pair-wise comparison tests indicated that observed differences between the urban schools were significant ($p < 0.05$).

Gene expression profiles can be affected by parasitic infections

The influence of parasitic infections on gene expression profiles, independent of area differences, was assessed by examining expression in the rural area only. Children with current *S. haematobium* infection had significantly lower CD14, LIR-7 and CD28 mRNA expression levels ($p < 0.05$) as shown in Figure 2. The mean expression levels of other genes involved in the TLR signalling pathway were lower among *S. haematobium* positives compared to negatives but these were not statistically significant. As expected, relative IgE mRNA expression showed the opposite trend and was higher

Table 1: Characteristics of the Study Population stratified by school

Factor	RURAL N = 48	URBAN		TOTAL N = 151
		Urban Low SES N = 47	Urban High SES N = 56	
Age*, mean (range), years	9.1 (6 - 13)	8.8 (6 - 12)	8.7 (5 - 13)	8.9 (5 – 13)
Gender, Male	22 / 48 (45.8%)	26 / 47 (55.3%)	30 / 56 (53.6%)	78 / 151 (51.7%)
Parasitic Infections n / N (%)				
S. haematobium positive a,b	26 / 48 (54.2%)	0 / 47 (0.0%)	0 / 56 (0.0%)	26 / 151 (17.2%)
Intestinal helminth positive** a,b,c	18 / 47 (38.3%)	5 / 37 (13.5%)	1 / 50 (2.0%)	24 / 134 (17.9%)
Malaria infection Positive *** a,b	24 / 46 (52.2%)	0 / 35 (0.0%)	1 / 55 (1.8%)	25 / 136 (18.4%)
Serology				
CRP (mg/ml), geometric mean (95%CI)§ a,b	3.0 (2.0 - 4.6)	0.6 (0.4 – 0.8)	0.9 (0.6 – 1.2)	1.1 (0.9 – 1.4)
Total IgE (IU/ml), geometric mean (95%CI) §§ a,b	5061.6 (3419.0 – 7491.3)	228.6 (157.4 – 332.9)	234.10 (159.6 – 343.3)	526.6 (383.7 – 722.8)

Abbreviations: CRP, C-reactive protein; CI, confidence interval; IgE, Immunoglobulin E; SES, socioeconomic status.
Results of Mann–Whitney and Pearson's $\chi 2$ Tests comparing variables of interest by area.
a: if P<0.05 for rural versus urban low SES.
b: if P<0.05 for rural versus urban high SES.
c: if P<0.05 for urban low SES versus urban high SES.
* Missing ages for 3 rural participants.
** Intestinal helminths detected were Hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*; missing intestinal helminth information for 1 rural, 10 urban low SES and 6 urban high participants.
*** Missing malaria information for 2 rural, 12 urban low SES and 1 urban high-SES participants.
§ Missing CRP values for 4 rural, 1 urban low SES and 3 urban high SES participants.
§§ Missing total IgE values for 11 rural and 1 urban low SES participants.

among *S. haematobium* infected children compared to uninfected children. With respect to intestinal helminths, as shown in Figure S2 (Supplementary material), there was a tendency towards lower expression levels of some of the TLR signalling genes among intestinal helminth infected participants compared to uninfected but this did not reach statistical significance. This tendency was observed specifically for NOD-2 (p = 0.09) and SOCS-3 (p=0.07). Malaria infection was not significantly associated with the expression levels of the genes investigated (Figure S3 – Supplementary material).

IL-10 gene expression in the rural area was not associated with current parasitic infection

After adjusting for age and gender, no significant associations were observed between IL-10 gene expression in the rural area and *S. haematobium* infection (p=0.292), having any intestinal helminth (p=0.967) or malaria infection (p=0.728).

Urban High vs. Urban Low				+	+	+		+	+			+		
Urban High vs. Rural	+		+	+	+	+	+	+		+	+		+	+
Urban Low vs. Rural	+											+		+

Figure 1: Relative gene expression profiles in urban high SES, urban low SES and rural schoolchildren
The relative gene expression profiles in each area expressed as z-scores with standard errors. Analysis of covariance models with individual mRNA expression levels as outcomes adjusted for age and gender were used to generate estimated marginal mean expression levels in each area.
+ = if $p < 0.05$ for the Bonferroni post-hoc pairwise comparison of estimated marginal mean gene expression levels between the 3 areas.

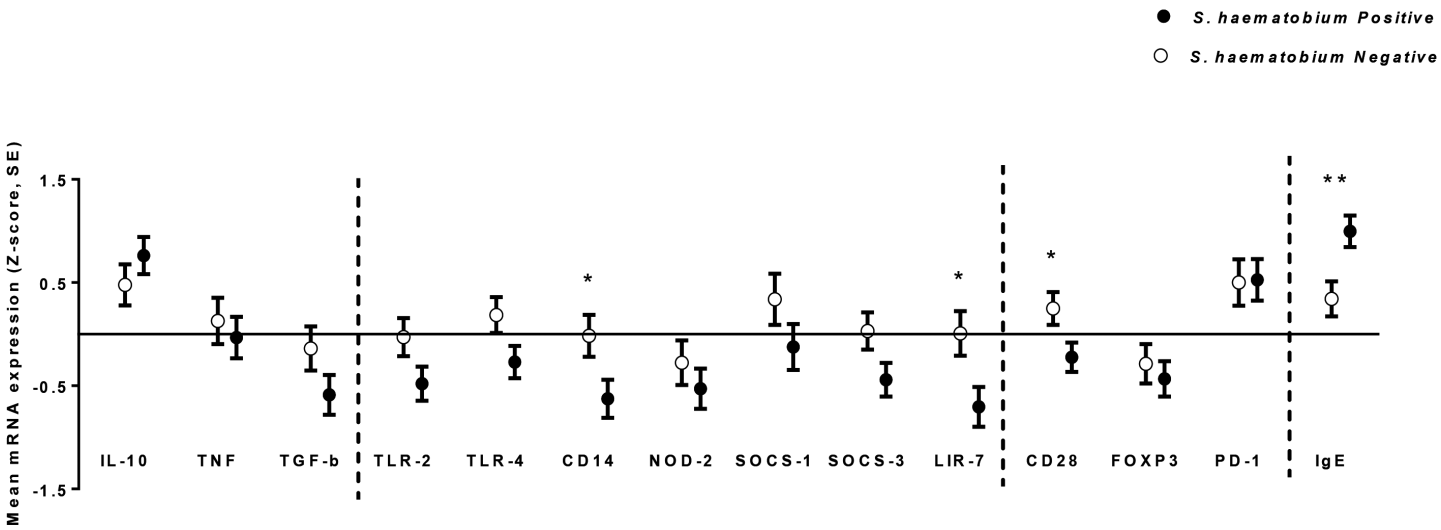


Figure 2: Relative expression profile in rural children stratified by *S.haematobium* infection status

The relative gene expression profiles in the rural area expressed as z-scores with standard errors stratified by *S. haematobium* infection status (positive versus negative). Analysis of covariance models with individual mRNA expression levels as outcomes adjusted for age, gender, intestinal helminth and malaria infection were used to generate estimated marginal means.

** P <0.01, * P <0.05 for analysis of covariance model test of between subject effects.

Area differences in IL-10 gene expression are not fully accounted for by IL-10 polymorphisms

Given the striking differences observed in relative IL-10 mRNA expression between rural and urban schoolchildren, we examined whether underlying variations in genetic polymorphisms could account for these differences.

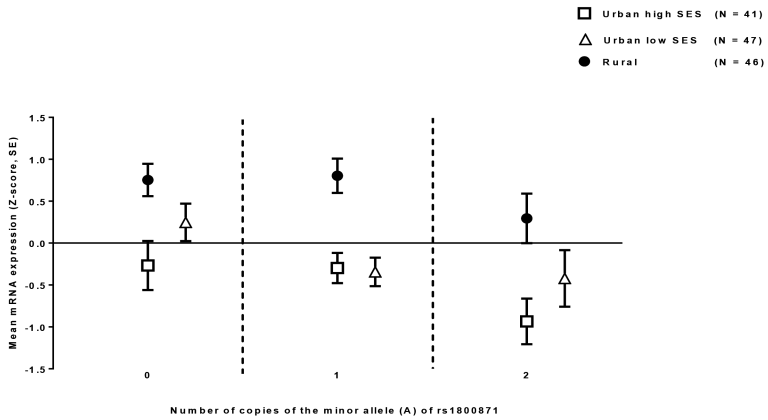
The minor allele frequencies (MAFs) for selected *IL-10* SNPs genotyped are shown in Table S3 (Supplementary material) and were compared to the Yoruba in Ibadan, Nigeria (YRI) genotype data from HapMap (www.hapmap.org) as well to data from a population study conducted in Northern Ghana [24]. As shown in Table S3, MAFs for the polymorphisms examined were similar across the three populations.

Table S4 details the MAFs for the *IL-10* SNPs stratified by area as well as the results of comparative between-area Pearson's χ^2 tests. Some significant differences in MAFs between the three areas were observed. Specifically, the MAF for rs3024496 was significantly higher in the rural school compared to both urban schools and the MAF for rs1878672 significantly lower in the urban high SES school compared to the other schools. In addition, the MAF for rs1800890 was significantly lower in the urban high SES school compared to rural school but not the urban low SES school.

Associations between *IL-10* SNPs and IL-10 mRNA expression were also examined for subjects with data for both parameters (N=134) and the results are shown in Table S5 (Supplementary material). After adjusting for area, only the marker rs1800871 was significantly associated with IL-10 mRNA ($p < 0.05$) with increasing copies of the minor allele of this SNP corresponding to decreasing IL-10 mRNA expression (shown in Figure S4 – Supplementary material). Figure 3 shows IL-10 mRNA expression in rural, urban low SES and urban high SES children according to the number of copies of the minor allele of *IL-10* SNP rs1800871. For rural children with two copies of the minor allele of rs1800871, IL-10 mRNA was higher compared to urban low SES and urban high SES children. When we examined the association between area and IL-10 mRNA after adjusting for the SNP rs1800871 and demographic factors, living in the rural area was still strongly associated with elevated IL-10 mRNA ($p = 1.87 \times 10^{-7}$).

Area differences in IL-10 gene expression are not explained by differences in ethnicity

Given that a few significant area differences in *IL-10* SNP frequencies were observed which may reflect variations in underlying genetics between the areas, we analyzed whether differences in reported ethnicity among the three groups could explain IL-10 gene expression variability. Information on ethnicity was available for 85 out of 151 children. For this subset, there was no association between reported ethnicity and IL-10 mRNA expression ($p=0.469$) after adjusting for age, gender and area. In this adjusted model, rural area was still strongly associated with elevated IL-10 mRNA expression ($p= 0.001$).



Urban High vs. Urban Low			
Urban High vs. Rural	+	+	+
Urban Low vs. Rural		+	

Figure 3: Relative IL-10 gene expression and copies of IL-10 SNP rs1800871

The relative IL-10 gene expression in urban high SES, urban low SES and rural children stratified by genotypes of *IL-10* SNP rs1800871.

+ = if p <0.05 for the Bonferroni post-hoc pairwise comparison of estimated marginal mean IL-10 gene expression levels between the 3 areas.

Area differences in TLR-2 and TLR-4 gene expression profiles are not explained by TLR-2 and TLR-4 polymorphisms

The minor allele frequencies for *TLR-2* and *TLR-4* SNPs in our population compared to the YRI genotype data from HapMap as well as to a population in Northern Ghana [25] are shown in Table S6 (Supplementary material). The MAFs across these three populations were comparable overall.

The area differences in minor allele frequencies for *TLR-2 and TLR-4* SNPs are shown in Table S7 (Supplementary material). For the *TLR-2* marker rs3804100, there were significant area differences but the overall MAF was low in our population. A significant difference was also observed for the marker rs4696480 which was significantly higher in the urban high SES school compared to urban low SES school. For *TLR-4* markers, the MAF of rs2737190 was significantly different between rural and urban high SES children (p=0.048) but the overall MAF of this SNP was low in our population. For rs10759932, there was a significant difference in MAF between rural and urban high SES children (p=0.036). A total of 142 children had data for TLR polymorphisms and TLR mRNA. None of the *TLR-2* and *TLR-4* markers were significantly associated with TLR-2 and TLR-4 mRNA expression, respectively (Table S8 - Supplementary material). In addition, adjusting for the *TLR-2* and *TLR-4* SNPs did not make a difference to observed area differences in mean TLR-2 and TLR-4 mRNA expression levels, respectively.

Discussion

In our study, we observed marked differences in the gene expression profiles of Ghanaian children attending schools in rural, urban low SES and urban high SES areas in the Greater Accra Region of Southern Ghana. Our study demonstrates that environmental determinants associated with specific geographical locations and lifestyle, have a strong impact on shaping immune gene expression profiles. Similar observations were made by Idaghadour *et al.* [14, 15] who used whole-genome expression arrays and found a genome-wide expression signature of regional population differences in Morocco. However, in our investigation, we further examined the effects of specific infections and socioeconomic differences on the gene expression patterns of our study population.

Higher gene expression levels of IgE, IL-10 and PD-1 were seen in the peripheral blood of rural compared to urban children in our study population. *S. haematobium* infection could account for the urban-rural difference observed in IgE gene expression as this infection in the rural area was strongly associated with higher IgE mRNA expression. This would be expected since high serum IgE protein is an established hallmark of schistosomiasis infection [29]. In addition, a publication by Hartgers *et al.* validated *ex vivo* mRNA expression in rural Ghana by showing a strong correlation between IgE mRNA and serum IgE [21].

Interestingly, current helminth infections did not account for the markedly elevated IL-10 and PD-1 mRNA levels among rural compared to urban children. For IL-10 this was somewhat unexpected since chronic helminth infections are characterized by an anti-inflammatory environment marked by elevated IL-10 and TGF-β [19]. Given this anomaly, we went on to examine whether differences in ethnicity or genetic polymorphisms may explain elevated IL-10 mRNA among rural compared to urban children. In a subset, no association was observed between ethnicity and IL-10 gene expression. Regarding genetic variants, while the minor allele of one particular marker rs1800871 was strongly associated with decreased expression of IL-10 mRNA, after controlling for the effects of this SNP, IL-10 mRNA expression in the rural area was still elevated compared to the two urban areas. This suggests that although underlying genetics plays a role in gene expression profiles, environmental factors appear to have a dominant influence. The lack of association between helminth infection and IL-10 mRNA expression might be due to the fact that in our study we have looked at current helminth infection whereas past infections could also have shaped the regulatory network. An additional factor to note is that the method used to diagnose helminth infections, might not have been sensitive enough to detect all infected subjects. Moreover, post-transcriptional regulation of the IL-10 gene has been reported [30] and therefore IL-10 gene expression might be different from the production of the protein. However, the possibility that factors other than helminth infections, such as environmental mycobacteria [31] play an important role in expanding IL-10 gene expression, cannot be ruled out and would need further investigation.

The level of IL-10 mRNA expression was also positively correlated with PD-1 gene expression. PD-1 protein is thought to have regulatory functions inhibiting T-cell proliferation and cytokine production [32]. In addition, PD-1 receptor is a well-established marker of 'T-cell exhaustion' which is the progressive loss of T-cell function under conditions of antigen persistence following chronic infections such as viral infections [33]. The role of IL-10 in T-cell exhaustion has also been demonstrated in the murine lymphocytic choriomeningitis virus system [34]. The correlation between IL-10 mRNA and PD-1 mRNA in our rural environment could indicate T-cell exhaustion in this area resulting from a chronic persistent infection.

We observed that the expression of TGF-β and FOXP3 was higher in the peripheral blood of children in both urban areas compared to their rural counterparts. A significant positive correlation between TGF-β and FOXP3 gene expression levels was also observed. The higher expression of these genes in the urban areas relative to the helminth-endemic rural area was unexpected since both TGF-β and FOXP3 are thought to be up-regulated as part of the immune regulatory network associated with chronic infections [35]. In addition, regulatory T cells expressing FOXP3 driven by TGF-β have been implicated in the suppression of host immunity during chronic helminth infection [36, 37]. It would be important to examine whether these molecules show higher expression in urban areas at the protein level as well.

The other set of genes with elevated expression in urban high SES subjects compared to the other two groups were some of those involved in TLR signalling. Specifically, TLR-2, TLR-4, CD14, NOD-2, SOC-3 and LIR-7. Of interest, studies in European farmers have indicated that higher exposure to microorganisms might be associated with higher expression of receptors such as TLR-2 and CD14 [38]. Here, we observed that *S. haematobium* infection in the rural area could explain the lower expression of CD14 and LIR-7 genes among the rural children and in comparison to the two urban groups. Other studies have shown that *Schistosoma* egg antigen down-regulates the expression of genes involved in the TLR signalling pathway [39] and we have previously reported lower TLR-2 mRNA expression associated with current *S. haematobium* infection in rural Ghanaian children [21]. The contrast between the effects of an environment rich in microorganisms or parasites on the expression of pattern recognition receptors (PRRs) in Europe versus in Africa might be explained by the very different types and burden of microorganisms and parasites that are present in these environments. Thus, in rural Ghana, exposures might lead to down-regulation whereas in central European farms, to the up-regulation of PRRs. Moreover, specific lifestyle factors may have a suppressive effect on the expression of genes involved in interactions with PAMPs within our population. None of the *TLR-2* or *TLR-4* SNPs examined was significantly associated with increased TLR-2 or TLR-4 mRNA expression respectively. Polymorphisms of *TLR* genes are of particular importance given the key role of TLRs as PRRs in host defence mechanisms against microbial pathogens [40].

Overall, we observed that not only were the rural and urban areas different but that there were also significant differences within the urban area. Changes along a gradient

from rural to urban low SES to urban high SES implicate factors that are likely to reflect exposure to pathogens. However, factors that segregate into urban (irrespective of SES) and rural area are likely to reflect characteristics of urbanization for example, changes in diet or pollution.

One limitation of our current study was that it was conducted with relatively small numbers of subjects in each study area. A larger sample size may have meant greater statistical power in detecting area differences for some of the genes as evident in some of the borderline significant observations. However, post-hoc power analysis based on mean IL-10 mRNA levels in urban compared to rural children showed the study to be sufficiently powered. Another weakness of our study was that there was only a single sample collected for parasitic infections. Therefore, there is the possibility that if infections were missed, we underestimated the prevalence of our parasitic infections. Although the focus of our genetic polymorphism selection was on variants of importance in Caucasian populations, studies conducted in Northern Ghana that included most of the genetic variants at the IL-10 gene locus examined in our study, demonstrated the functional importance of these variants in a Ghanaian population [24, 41]. Specifically, the minor allele of rs1800871 that was negatively associated with IL-10 mRNA in our study was negatively associated with *ex vivo* IL-10 cytokine production in response to stimulation with *E. coli* lipopolysaccharide and *Saccharomyces cerevisiae* zymosan in these two studies [24, 41]. However, the possibility still exists that IL-10 polymorphisms that were not examined in our study, may contribute to the observed rural versus urban differences in the expression of IL-10 mRNA.

Common to all mRNA studies of whole blood, our study suffers from the fact that mRNA expression might not be directly related to protein expression levels. Although we used IgE as a positive control, showing that mRNA expression was paralleled by protein levels, this might not be the case for all genes examined. In addition, the expression of the mRNA is in whole blood and does not reveal any cell-specific profiles which might be important when considering their function in determining disease profiles. An additional weakness of our investigation is that differential blood cell counts were not assessed and differences in cellular composition may play a role in the expression patterns observed.

Despite the limitations, our study demonstrates that contrasting environments shaped by urbanization and associated characteristics contribute significantly to gene expression profiles among children. Future studies are needed to identify specific factors that activate particular immunological pathways and to understand the functional consequences of the differential gene expression profiles observed in terms of disease patterns and susceptibility.

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Conflict of interest

The authors declare no conflict of interest.

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Table S1: Description of genes*

	GENE		
CATEGORY	**ABBREVIATION**	**FULL NAME**	**FUNCTION OF PROTEIN**
Cytokines	IL-10	Interleukin 10	Cytokine with multiple effects in immune regulation and inflammation.
	TNF	Tumor necrosis factor	Multifunctional pro-inflammatory cytokine
	TGF-*b*	Transforming growth factor beta	Immunosuppressive cytokine with multiple regulatory functions
Toll Like Receptor Signalling	TLR-2	Toll-like receptor 2	Key receptor in pathogen recognition and activation of innate immunity.
	TLR-4	Toll-like receptor 4	Key receptor in pathogen recognition and activation of innate immunity.
	CD14	Cluster of differentiation 14	Preferentially expressed surface antigen that cooperates with other proteins to mediate the innate immune response to bacterial lipopolysaccharide
	NOD-2	Nucleotide-binding oligomerization domain containing 2	Intracellular pattern recognition receptor that recognizes bacterial muramyl dipeptide
	SOCS-1	Suppressor of cytokine signalling 1	Negative regulator of cytokines that signals through the JAK/STAT3 pathway
	SOCS-3	Suppressor of cytokine signalling 3	Negative regulator of cytokines that signals through the JAK/STAT pathway
T-cell activation & polarization	LIR-7	Leukocyte immunoglobulin-like receptor	Receptor with immunosuppressive properties but also implicated in activation
	CD28	Cluster of Differentiation 28	Co-stimulatory molecule expressed on T-cells that provides signals for T-cell activation
	FOXP3	Forkhead box P3	Master regulator in the function of regulatory T-cells
	PD-1	Programmed cell death 1	Receptor with inhibitory functions known as a potent regulator of immune responses
Antibody	IgE	Immunoglobulin E	Antibody key in allergic responses and helminth infections

*Adapted from van Riet E *et al.,* Combined TLR2 and TLR4 ligation in the context of bacterial or helminth extracts in human monocyte derived dendritic cells: molecular correlates for Th1/Th2 polarization. *BMC Immunology 2009*; 10(1):9.

Table S2: Results of Bonferroni pair-wise comparisons of between area differences in gene expression levels

	Between Area Bonferroni Pair-wise Comparisons									ANCOVA Test of Between-Subject Effects for Area and Gene Expression (Outcome)
	Urban High SES (I) vs Urban Low SES (J)			Urban High SES (I) vs Rural (J)			Urban Low (I) vs Rural (J)			
GENE	Mean difference (I – J)	Std. Error	p-value	Mean difference (I – J)	Std. Error	p-value	Mean difference (I – J)	Std. Error	p-value	p-value
IL-10	-0.34	0.17	0.152	-1.12	0.17	**5.73×10^{-9}**	-0.78	0.18	**1.08×10^{-4}**	**9.53×10^{-9}**
TNF	0.45	0.19	0.069	0.20	0.20	0.968	-0.25	0.21	0.671	0.074
TGF-b	0.40	0.19	0.108	0.79	0.19	**2.27×10^{-4}**	0.39	0.20	0.172	**3.69×10^{-4}**
TLR-2	0.89	0.18	**9.21×10^{-6}**	0.86	0.19	**2.33×10^{-5}**	-0.03	0.19	1.000	**7.45×10^{-7}**
TLR-4	0.95	0.18	**2.60×10^{-6}**	0.53	0.19	**0.015**	-0.41	0.19	0.106	**4.58×10^{-6}**
CD14	0.70	0.17	**3.07×10^{-4}**	0.92	0.18	**2.32×10^{-6}**	0.22	0.18	0.704	**1.09×10^{-6}**
NOD-2	0.63	0.19	**0.003**	0.77	0.19	**2.05×10^{-4}**	0.14	0.20	1.000	**9.01×10^{-5}**
SOCS-1	0.22	0.20	0.805	0.03	0.20	1.000	-0.19	0.21	1.000	0.504
SOCS-3	0.87	0.19	**2.10×10^{-5}**	0.73	0.19	**4.82×10^{-4}**	-0.13	0.20	1.000	**7.20×10^{-6}**
LIR-7	0.90	0.18	**4.62×10^{-6}**	0.99	0.18	**7.80×10^{-7}**	0.09	0.19	1.000	**5.52×10^{-8}**
CD28	0.19	0.20	1.000	0.11	0.20	1.000	-0.08	0.21	1.000	0.634
FOXP3	-0.01	0.19	1.000	0.56	0.20	**0.016**	0.58	0.21	**0.018**	**0.007**
PD-1	-0.45	0.18	**0.049**	0.89	0.19	**1.34×10^{-5}**	-0.45	0.20	0.071	**2.48×10^{-5}**
IgE	0.01	0.18	1.000	0.95	0.18	**2.20×10^{-6}**	-0.963	0.19	**3.91×10^{-6}**	**2.29×10^{-7}**

P-values <0.05 are shown in bold.

Table S3: *IL-10* Polymorphisms (N=330)

Marker	Chromosome Position	Gene Location	Alleles*	Minor Allele Frequency			Hardy Weinberg p-value (current study)
				Current Study Population	Northern Ghana Study Population **	Yoruba in Ibadan (YRI) ***	
rs3024498	1: 206941529	Exon	T/C	0.085	0.083	0.092	0.052
rs3024496	1: 206941864	Exon	A/G	0.399	0.425	0.371	0.142
rs1878672	1: 206943713	Intron	G/C	0.233	0.244	0.235	0.209
rs1800871	1: 206946634	Promoter	G/A	0.398	0.470	0.466	0.261
rs1800893	1: 206947167	Promoter	C/T	0.324	0.284	0.303	**0.001**
rs1800890	1:206949365	Promoter	A/T	0.220	0.201	0.204	0.894
rs12122923	1:206951397	Promoter	C/T	0.123	n.a.	0.131	0.252
rs10494879	1:206952204	Promoter	C/G	0.285	0.284	0.255	0.201

*Minor allele underlined.
IL10 Minor allele Frequencies from a study conducted in a rural community in Northern Ghana (Kuningas M *et al*. Selection for genetic variation inducing pro-inflammatory responses under adverse environmental conditions in a Ghanaian population. *PLOS ONE* 2009; **4(11)**:** e7795), N= 4336.
***Minor allele Frequencies from **HapMap Database Release Number 28 PhaseII+III, August 2010 dbSNP b126.**
n.a.: Information not available.
P-values <0.05 are shown in bold.

Table S4: Minor allele frequencies of *IL-10* Polymorphisms stratified by area (N=330)

Marker	Minor Allele	Minor allele frequencies by Area			Pearson's χ^2 Test p-value for comparisons between areas		
		Urban High SES (N=109)	Urban Low SES (N=123)	Rural (N= 98)	Urban High SES vs. Urban Low SES	Urban High SES vs. Rural	Urban Low SES vs. Rural
rs3024498	C	0.084	0.087	0.082	0.922	0.999	0.925
rs3024496	G	0.305	0.392	0.505	0.058	**4.97×10^{-5}**	**0.018**
rs1878672	C	0.158	0.258	0.276	**0.012**	**0.005**	0.676
rs1800871	A	0.435	0.409	0.347	0.595	0.083	0.190
rs1800893	T	0.319	0.323	0.331	0.933	0.804	0.860
rs1800890	T	0.165	0.240	0.253	0.053	**0.032**	0.756
rs12122923	T	0.106	0.103	0.165	0.925	0.088	0.058
rs10494879	G	0.225	0.307	0.321	0.052	0.035	0.769

P-values <0.05 are shown in bold.

Table S5: *IL-10* Polymorphisms and IL-10 mRNA production (adjusted for age, gender and area)

IL-10 Marker name	Estimate (β)	Standard Error (SE)	IL-10 mRNA Linear Trend (p-value)
rs3024498	0.09	0.20	0.638
rs3024496	0.05	0.10	0.611
rs1878672	0.17	0.12	0.164
rs1800871	-0.28	0.11	**0.013**
rs1800893	0.20	0.11	0.084
rs1800890	0.14	0.13	0.277
rs12122923	0.04	0.15	0.776
rs10494879	0.16	0.12	0.172

P-values <0.05 are shown in bold.

Table S6: *TLR* Polymorphisms (N=318)

					Minor Allele Frequency			Hardy Weinberg p-value (current study)
Gene	Marker	Chromosome Position	Gene Location	Alleles*	Current Study Population	Northern Ghana Study Population **	Yoruba in Ibadan (YRI) ***	
TLR-2	rs3804099	4:154624656	Exon	C/T	0.374	0.380	0.364	0.131
	rs3804100	4:154625409	Exon	T/C	0.038	0.032	0.054	0.484
	rs4696480	4:154607126	Promoter	T/A	0.358	n.a.	0.000	0.120
TLR-4	rs4986790	9:120475302	Exon	A/G	0.094	0.075	0.041	0.392
	rs2737190	9:120464181	Promoter	G/A	0.094	n.a.	0.143	0.884
	rs10759932	9:120465144	Promoter	T/C	0.312	0.267	0.258	0.060
	rs4986791	9:120475602	Exon	C/T	0.009	0.012	0.022	0.865

*Minor allele underlined.
****** *TLR-2* and *TLR-4* gene minor allele frequencies from a study conducted in a rural community in Northern Ghana (May L *et al*. Polymorphisms in TLR-4 and TLR-2 genes, cytokine production and survival in rural Ghana. *European journal of human genetics* 2010; **18**(4)**:** 490-5), N= 4292.
******* Minor allele Frequencies from **HapMap Database Release Number 28 PhaseII+III, August 2010 dbSNP b126.**
n.a.: Information not available.

Table S7 Minor allele frequencies of TLR-2 and TLR-4 SNPs stratified by Area (N=318)

			Minor allele frequencies by Area			Pearson's χ^2 Test p-value for comparisons between areas		
Gene	Marker	Minor Allele	Urban High SES (N=115)	Urban Low SES (N=113)	Rural (N= 90)	Urban High SES vs. Urban Low SES	Urban High SES vs. Rural	Urban Low SES vs. Rural
TLR-2	rs3804100	T	0.065	0.027	0.017	**0.049**	**0.017**	0.502
	rs3804099	C	0.382	0.332	0.417	0.269	0.472	0.079
	rs4696480	A	0.400	0.308	0.367	**0.041**	0.491	0.214
TLR-4	rs4986790	G	0.089	0.089	0.106	0.985	0.574	0.582
	rs2737190	A	0.128	0.080	0.068	0.096	**0.048**	0.646
	rs10759932	C	0.271	0.306	0.369	0.409	**0.036**	0.186
	rs4986791	T	0.009	0.009	0.011	1.000	0.819	0.819

P-values <0.05 are shown in bold

Table S8: TLR Polymorphisms and TLR mRNA production (adjusted for age, gender and area)

Gene	Marker	Estimate (β)	Standard Error (SE)	TLR-2 mRNA Linear Trend p-value
TLR-2	rs3804099	0.084	0.123	0.496
	rs3804100	-0.456	0.292	0.120
	rs4696480	-0.032	0.119	0.790
Gene	**Marker**	**Estimate (β)**	**Standard Error (SE)**	**TLR-4 mRNA Linear Trend (p-value)**
TLR-4	rs4986790	-0.07	0.190	0.715
	rs2737190	-0.37	0.210	0.080
	rs10759932	0.033	0.128	0.795
	rs4986791	1.183	1.009	0.243

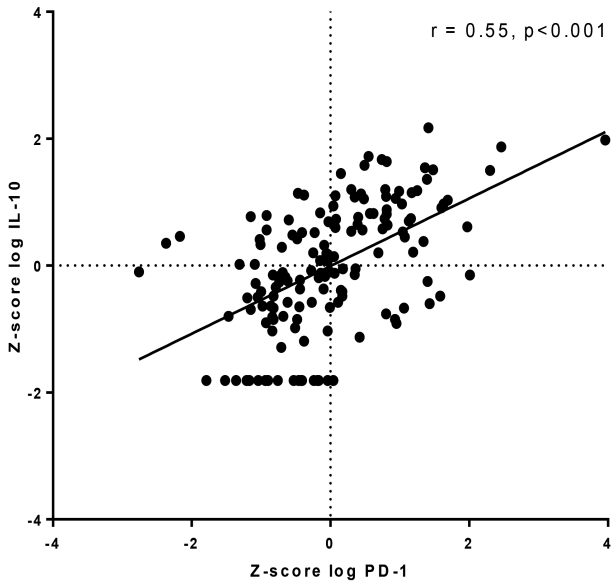


Figure S1: Correlation between IL-10 mRNA and PD-1 mRNA expressed as z-scores

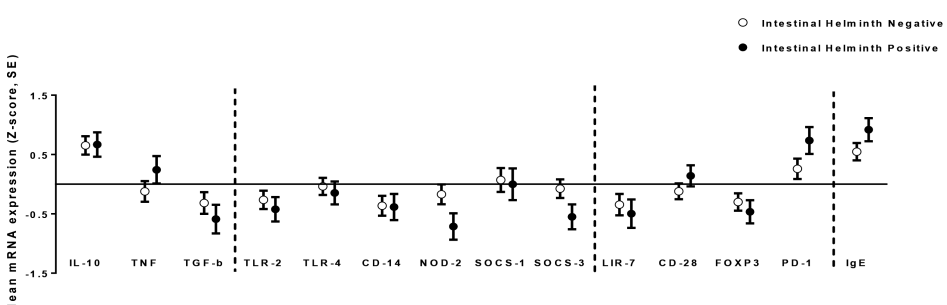


Figure S2: Relative gene expression profile in rural children stratified by intestinal helminth infection status (N=47)

The relative gene expression profiles in the rural area expressed as z-scores with standard errors stratified by intestinal helminth infection status (positive versus negative). Analysis of covariance models with individual mRNA expression levels as outcomes adjusted for age, gender and other parasitic infections were used to generate estimated marginal mean expression levels.

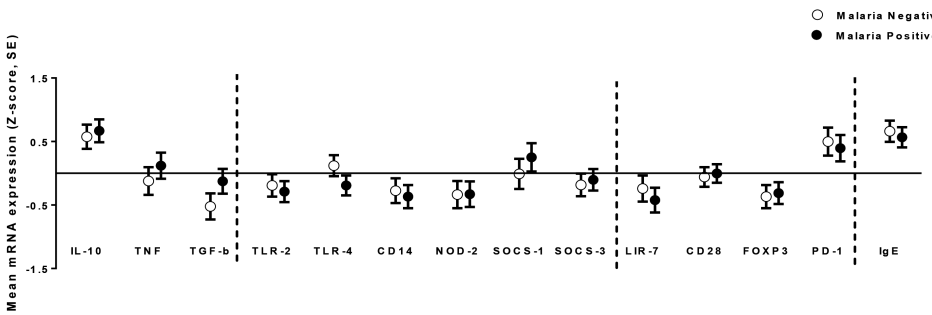


Figure S3: Relative gene expression profile in rural children stratified by malaria infection status (N=47)

The relative gene expression profiles in the rural area expressed as z-scores with standard errors stratified by malaria infection status (positive versus negative). Analysis of covariance models with individual mRNA expression levels as outcomes adjusted for age, gender and other parasitic infections were used to generate estimated marginal means.

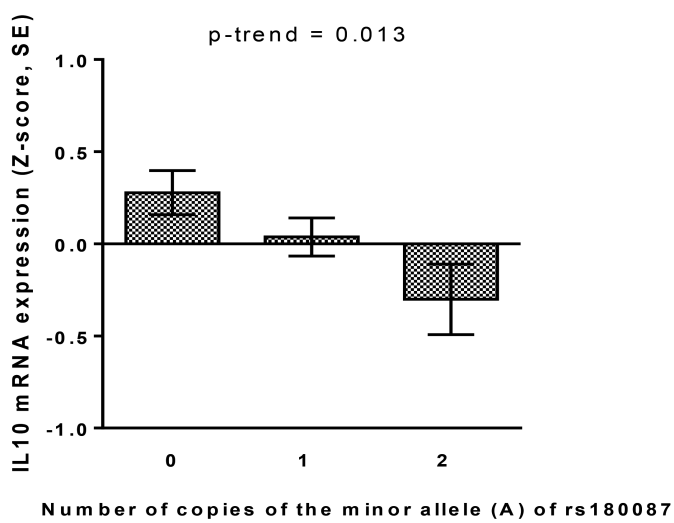


Figure S4: IL10 mRNA expression according to number of copies of marker rs1800871

The correlation between increasing copies of the minor allele of IL-10 SNP rs1800871 and IL-10 mRNA (expressed as a z-score with standard errors). An analysis of covariance model with IL-10 mRNA expression level as an outcome adjusted for age, gender and area was used to generate estimated marginal mean levels according to number of copies of the IL-10 SNP.

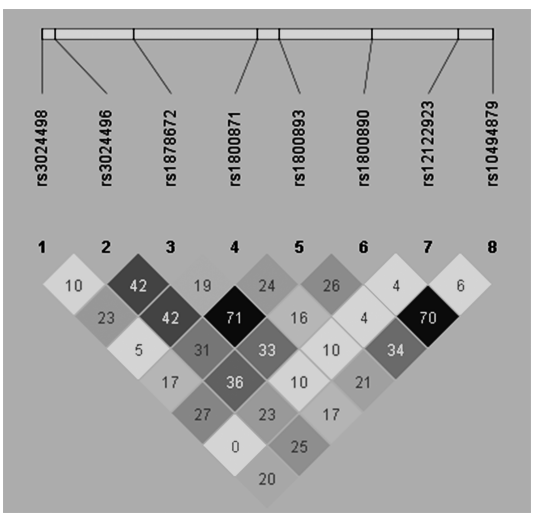
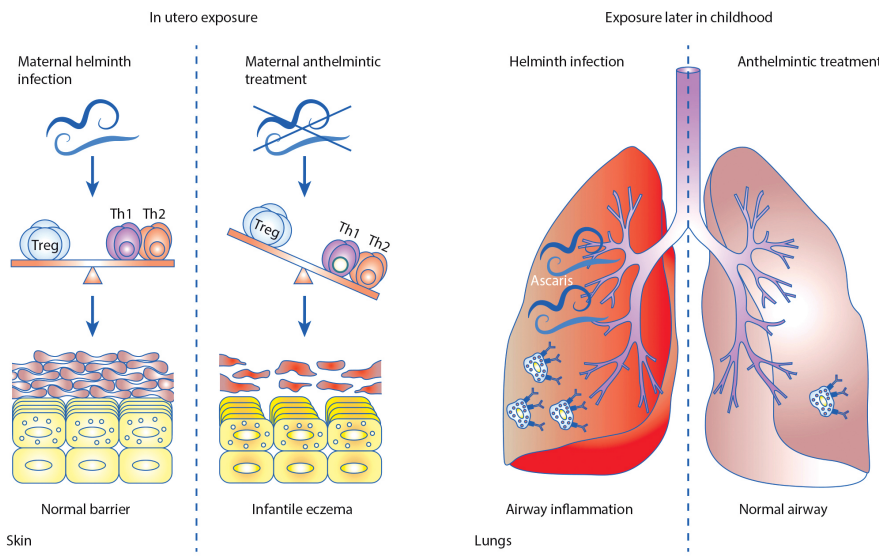


Figure S5: Linkage disequilibrium plot of IL-10 SNPs

The figure shows the linkage disequilibrium plot of IL-10 SNPs genotyped in the study and their relative positions. The increasing strength of correlation between SNPs is indicated from white to dark grey.



Discovering how anthelmintic treatment influences allergic symptoms in early and late childhood
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chapter 7

Parasitic worms and allergies in childhood: insights from population studies 2008-201

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Abstract

The last few decades have seen a marked increase in the global prevalence of allergic diseases particularly among children. Among the factors attributed to this rise has been reduced exposure to pathogens during childhood leading to insufficient maturation of the regulatory arm of developing immune systems. Over the years, a number of epidemiological studies have observed an inverse relationship between parasitic worm (helminth) infections and allergies. The purpose of this review is to highlight insights from population studies conducted among children published between 2008 and 2013 that explore the complex dynamics between helminth infections and allergies. These insights include the effect of anthelmintic treatment on allergic responses, an elucidation of immune mechanisms and an examination of helminth-induced immunoglobulin E cross-reactivity.

A better understanding of the relationship between helminths and allergies is imperative as research directions move towards harnessing the therapeutic potential of helminths and their products in the treatment of allergic disorders.

Keywords

Anthelmintic treatment; asthma; atopy; eczema; helminths; immune mechanisms; immunoglobulin E cross-reactivity; rhinoconjunctivitis; skin prick testing; urbanization

Introduction

Over the past few decades, there has been a sharp increase worldwide in the prevalence of allergic disorders such as asthma, rhinitis, eczema and food allergies particularly among children [1]. The hygiene hypothesis provides an explanation for these observations in terms of how over the course of time in Western countries, improved hygiene, smaller family sizes and fewer childhood infections may have driven an increase in allergies [2]. In immunological terms, reduced pathogenic exposure during childhood leads to inadequate maturation of the immune system's regulatory arm thus resulting in uninhibited inflammatory responses towards harmless antigens [3, 4].

A rise in allergic diseases is also being observed in rapidly urbanizing developing countries where a reduction in infectious diseases, improved hygiene and the adoption of a so-called "western lifestyle" are factors driving this increase [5]. For example, studies from Asian economic hubs dating back to the 1970s illustrate how a higher prevalence of asthma among urban populations was associated with wealth and lifestyle in contrast with a lower prevalence of asthma in rural environments [6]. These investigations emphasize the importance of environmental exposures in the pathogenesis of allergic disorders in general. Key among such exposures is infections with parasitic worms (helminths).

Helminths are metazoan parasites that have evolved the ability to down-regulate their host's immune responses and thus protect against their own elimination as well as reduce severe pathology in the host [7, 8]. Over 1 billion people worldwide are infected with one or more helminth species [9]. Most of these individuals are currently found in tropical regions of the world where such infections are linked to poverty and poor sanitation [10].

Helminth infections are of particular interest to studies of allergic disorders as they induce strong T-helper-2 (Th2) responses leading to high levels of immunoglobulin E (IgE) antibodies. Despite the common immunological profiles associated with both helminths and allergies, there is little overlap in the geographical distribution of these two health problems. This suggests that not all Th2 responses lead to allergic outcomes and that strong Th2 profiles seen in helminth-infected subjects do not translate into allergic disease. In fact, studies show that helminth infections could even be inversely associated with allergic disorders [11, 12]. However, the interactions are indeed complex as some investigations find an inverse association but others show no effect or even a positive association.

In recent years, studies among children from helminth-endemic areas at various stages of urbanization have provided new and interesting insights. The purpose of this review is to highlight research findings from population studies focused on children aged 0 to 18 years and published between 1 January 2008 and 31 July 2013. To identify relevant publications, searches were conducted in PubMed using key words related to 'allergy' or 'hypersensitivity' in combination with 'helminths', 'parasites' or 'worms'. These were restricted to human studies in children (birth – 18 years). Studies focusing on helminths that have not evolved to infect humans but where humans acquire infections by accident such as *Toxocara* species [13-16], *Echinococcus granulosus* [17] and *Ascaris suum* [18] were excluded.

Helminths and allergy: associations in population studies

Recent cross-sectional studies conducted in Brazil by the Social Changes, Asthma and Allergy in Latin America (SCAALA) group [19] have shown that among urban poor children aged 4-11 years living in Salvador, heavy infection with the helminth *Trichuris trichiura* in early childhood (on average at age 2 years) is associated with reduced odds of skin prick test (SPT) reactivity later in childhood [20]. Apart from demonstrating the importance of timing and early-life infections in the pathogenesis of childhood allergy, this study illustrates how heavy helminth infections (compared to light infections) may have a protective effect against allergies. Similarly, an investigation from South Africa comparing allergy outcomes among rural children of the Xhosa ethnicity to urban Xhosa children of low socioeconomic status found that after adjusting for area and detectable allergen-specific IgE, current *Ascaris lumbricoides* infection was associated with reduced odds of SPT reactivity [21].

However, other investigations have observed no effect of helminths on SPT reactivity. For example, among Cuban children aged 4-14 years living in helminth-endemic areas, current intestinal helminth infection was not associated with SPT reactivity [22]. However, it is important to note that among this study population, given the well-organized health provision in Cuba, it is very likely that the children were regularly dewormed and indeed the intensity of helminth infections was relatively low. Some studies have even observed a positive association between *Ascaris*-specific IgE (sIgE) and SPT reactivity for example, among urban black adolescents (median age 18 years) living in Cape Town, South Africa [23]. However, a limitation of the latter study is that *Ascaris*-sIgE may not be a useful marker for current ascariasis infection since elevated levels may indicate past infection or cross-reactivity due to other helminth antigens or environmental allergens.

Aside from SPT reactivity, the effects of helminths on other allergy outcomes have also been examined. Among a cohort of 3960 Afro-Ecuadorian children aged 6-16 years living in Ecuador, heavy *T. trichiura* infection was inversely associated with atopic wheeze but not with non-atopic wheeze [24]. On the other hand, a case-control study among 219 5 year old rural Bangladeshi children reported that current *A. lumbricoides* infection was not significantly associated with reported wheeze [25].

With regards to airway hyperresponsiveness, Calvert and Burney examined urban and rural Xhosa children and observed that current *A. lumbricoides* infection was associated with increased odds of exercise-induced bronchoconstriction (EIB) [21]. Since they had also found an inverse association between *A. lumbricoides* and SPT reactivity, they concluded that in areas with a heavy burden of *A. lumbricoides* infection, this helminth may induce an inflammatory response in the lungs that is independent of the parasite's effect on SPT reactivity [21]. In line with this, in a case-control study design, children with heavy *A. lumbricoides* infection (>100 eggs / grams) in Brazil, were found to be five times more likely to have bronchial hyperresponsiveness (BHR) measured by bronchial provocation tests compared to children with low loads or no infection [26].

When it comes to reported allergic disease, current infection with *A. lumbricoides* was linked to a more than 4 times reduced odds of atopic eczema in rural Cuban children aged 4-14 years [27]. Conversely, a history of *Enterobius vermicularis* infection in the same children was associated with an increased risk of reported allergic rhinoconjunctivitis and atopic eczema, emphasizing the importance of taking into consideration species of helminth. A history of hookworm infection was also associated with reported allergic rhinoconjunctivitis [27]. However, given that reported history of helminth infection can be an unreliable parameter, these findings should be considered with caution.

Aside from just helminths, some investigations have looked at multiple infections with other childhood pathogens. For example, among the SCAALA cohort in Salvador, Brazil, a cross-sectional study investigated whether helminth, viral and bacterial infections were associated with reported wheeze, SPT reactivity and specific IgE to locally important allergens. The study observed that in addition to the protective effect of *A. lumbricoides* infection, past exposure to *Toxoplasma gondii*, Epstein - Barr virus and herpes simplex virus (assessed by seropositivity) were each associated with a lower prevalence of SPT reactivity but not reported wheeze [28]. This finding highlights the important role of diverse childhood pathogens in reducing the risk of SPT reactivity. Furthermore, a birth cohort of children from Ethiopia observed that at 3 years, *Helicobacter pylori* infection was linked to borderline reduced odds of reported eczema as well as SPT reactivity to house dust mite [29].

Taken together, although there is strong evidence for protective effects of helminths on allergic outcomes in animal models [8], the results of cross-sectional studies in humans vary. Though it is generally agreed that helminth infections are often negatively associated with SPT, no or positive associations are reported with lung function or reported clinical symptoms of allergy. It is important to bear in mind that species of helminth as well as timing and burden of infection can all contribute to inconsistent findings in population studies particularly when the study outcome is as complex and multifactorial as clinical allergy.

Effect of anthelmintic treatment on allergy markers

Cross-sectional studies examining associations between helminths and allergy outcomes are prone to the problem of temporality [30] since these parameters are determined at the same time. Therefore, prospective studies are needed to fully investigate causality.

Some early longitudinal studies on the effect of repeated anthelmintic treatment among children in helminth-endemic areas observed an increase in SPT reactivity to aeroallergens in treated compared to placebo groups [31, 32] while another investigation found no effect [33]. Among studies published in the last 5 years, a randomized double-blind placebo-controlled trial on the effect of anthelmintic treatment among 1566 Vietnamese schoolchildren aged 6-17 years observed that after 12 months of anthelmintics at 0, 3, 6 and 9 months, treatment was associated

with an increased risk of SPT reactivity to any allergen [34]. However, the trial did not observe an effect of anthelmintic treatment on EIB, wheeze, rhinitis or flexural eczema. Also in Southeast Asia, a household-based cluster-randomized, double-blind place-controlled trial in a helminth-endemic area on Flores Island, Indonesia, assessed the effect of anthelmintic treatment (albendazole) every 3 months for 21 months on SPT reactivity among 1364 children aged 5-15 years [35]. At 21 months, treatment was associated with a statistically significant increase in the risk of SPT reactivity to cockroach allergen but not to 'any allergen' [35]. Similar to the trial in Vietnam, anthelmintic treatment had no effect on reported symptoms of asthma and atopic eczema in this study population [35].

Among 108 intestinal helminth positive Cuban schoolchildren aged 5-13 years, van der Werff *et al.* investigated the effect of anthelmintic treatment every 6 months for 24 months on SPT reactivity and reported allergic disease outcomes [36]. During the follow-up period, four groups of infected children from randomly selected primary schools in the same municipality as the treated cohort were used as reference groups to assess general trends over time in the outcomes of interest. The prevalence of SPT reactivity increased significantly following 1st and 2nd anthelmintic treatment but returned to the baseline prevalence subsequently [36]. The study observed that deworming was associated with a significant reduction in the proportion of children reporting asthma but not allergic rhinoconjunctivitis or atopic eczema [36].

Altogether, the majority of recent studies appear to suggest that anthelmintic treatment of at least one year increases SPT reactivity (Figure 1) but has no effect on reported allergic symptoms. As the prevalence of clinical symptoms is generally low, the question remains whether studies are sufficiently powered for these outcomes.

In terms of long-term anthelmintic treatment, a study in Ecuador in rural communities examined the impact of 15-17 years of community treatment with the anthelmintic drug ivermectin on the prevalence of SPT reactivity and allergic symptoms among schoolchildren aged 6-16 years [37]. The study found that the prevalence of SPT reactivity was two times greater among children living in treated communities compared to children living in adjacent untreated areas. Treatment was also associated with more than 2 times the odds of reported eczema but not symptoms of asthma and rhinoconjunctivitis [37].

The fact that most studies on the effect of anthelmintic treatment focus on school-age children has led to speculation that the lack of an effect of treatment on clinical allergy outcomes may be due to the age of children enrolled in these studies. By school-age, these children may have passed through the key windows in early life during which their immune systems are primed towards phenotypes that are more susceptible to or protected against allergies later in childhood. Therefore, studies among younger children in helminth-endemic countries are essential in furthering our understanding of how the developing immune system is protected against allergies at a young age.

One such study was a large randomized, double-blind, placebo-controlled trial carried out in Uganda that examined the effects of anthelmintic treatment among

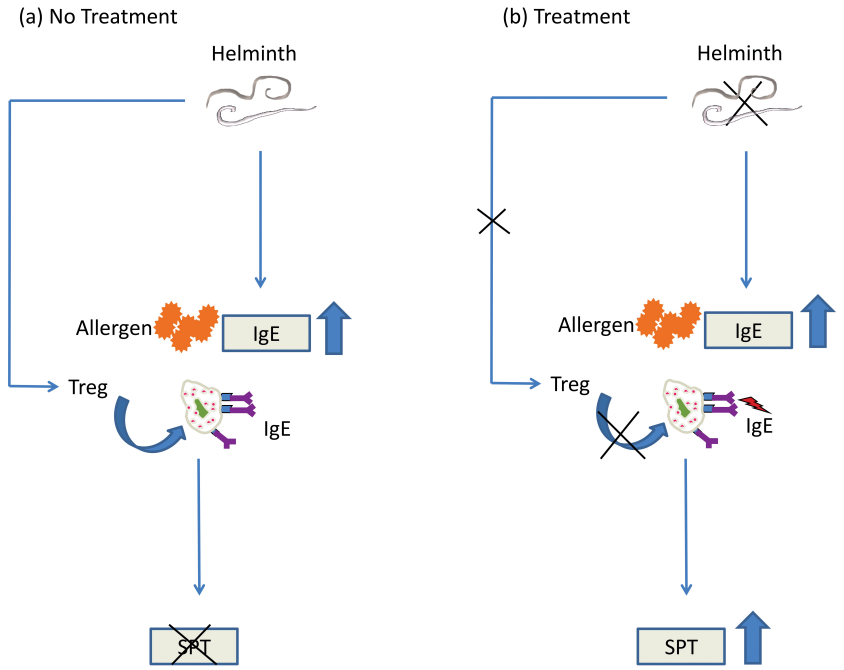

Figure 1: The effect of anthelmintic treatment on IgE sensitization and SPT reactivity

(a) During a chronic helminth infection, elevated levels of allergen-specific IgE are observed but helminth-induced regulatory mechanisms suppress SPT reactivity. (b) Following anthelmintic treatment and the removal of helminths, IgE memory remains largely unaffected but regulatory mechanisms decrease allowing increased SPT reactivity.

pregnant women on allergy outcomes in their offspring. In this trial, 2507 women in an area endemic for soil-transmitted helminths as well as the water-borne helminth *Schistosoma mansoni* were recruited and allocated to receive either albendazole (for soil-transmitted helminths) versus placebo or praziquantel (for *S. mansoni*) versus placebo. The trial found that treatment with albendazole (compared to placebo) among pregnant women was strongly linked to an increased risk of doctor-diagnosed infantile eczema in their offspring [38]. Praziquantel treatment had no overall effect but among infants whose mothers were *S. mansoni* positive at baseline, praziquantel treatment was associated with an increased risk of doctor-diagnosed infantile eczema but had no effect among infants whose mothers were *S. mansoni* negative [38]. The trial also found that albendazole treatment was positively associated with reported recurrent wheeze.

Within the same Ugandan birth cohort, the offspring of the enrolled pregnant women were randomized to receive quarterly single-doses of albendazole or placebo from the age of 15 months to 5 years [39]. By 5 years, no effect of quarterly albendazole treatment on eczema was observed. However, this may not be surprising given that the prevalence of helminth infections was extremely low in this cohort.

Aside from helminth infections in endemic areas, a Danish study examined whether enterobiasis infection, common in Western Europe and North America [40], protects against chronic inflammatory diseases [41]. The study examined prescriptions for the anthelmintic medication mebendazole as a proxy for enterobiasis infection among 924,749 children. Mebendazole prescription information was linked to diagnoses of asthma from the age of 5 years onwards through the national patient registry. Filling a prescription for mebendazole was associated with a very small but significant increased risk of asthma [41].

Taken together, these studies suggest that worms in early life are able to lower the risk of developing clinical allergy, the most common in this time window being eczema, while this does not seem to apply to airway allergy, which often develops later in life (Figure 2).

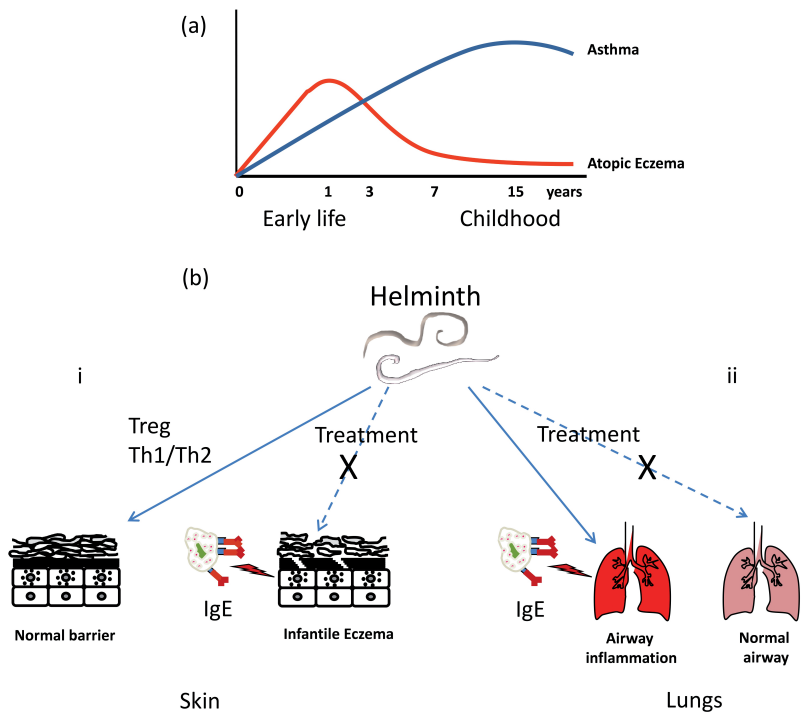

Figure 2: The effects of anthelmintic treatment on allergy-related symptoms in early life and later childhood.

Theoretical framework for the effects of anthelmintic treatment on allergy-related symptoms in early life and later childhood. (a) The current model of the Allergic March with atopic eczema peaking in the first years of life while asthma peaks later in childhood. (b-i) In pregnant women with helminth infections, *in utero* signals alter the immune system of the foetus resulting in a lack of early symptoms of allergy such as infantile eczema. However, treatment of pregnant women removes this immune modulatory activity and leads to increased manifestation of infantile eczema (b-ii) In later childhood, helminth infections either have no effect on allergy-related symptoms or in the case of *Ascaris lumbricoides* which has a lung stage, there is airway inflammation and symptoms of asthma. Anthelmintic treatment in *A. lumbricoides*-infected individuals would lead to the restoration of normal airway activity and fewer reports of asthma symptoms.

Immune mechanisms

Type 2 immune responses induced by helminths are characterized by the expansion of innate lymphoid cells-2 [42] as well as Th2 cells that lead to increased production of cytokines such as interleukin 4 (IL-4), IL-5, IL-9 and IL-13 [43]. During a helminth infection, these factors are all key to the control of inflammation, enhancement of tissue repair and can result in worm expulsion [44]. Moreover, chronic helminth infections can induce an immune regulatory network in the host characterized by regulatory T cells, regulatory B cells and alternatively activated macrophages [43]. The result is an anti-inflammatory environment typified by elevated levels of IL-10 and TGF-β as well as general T-cell hyporesponsiveness [8] which is thought to enhance survival of the worms within their immunocompetent host.

A number of studies in humans have provided evidence that IL-10 plays a key role in the helminth-induced immune regulation of allergic responses [8]. A study conducted in Gabon, Central Africa had established that children infected with the helminth *Schistosoma haematobium* had lower SPT reactivity to house dust mite compared to uninfected children in the same area [45]. The study showed that IL-10 production by parasite-antigen stimulated peripheral blood mononuclear cells (PBMCs) was higher in *S. haematobium* infected children compared to uninfected and elevated IL-10 levels were negatively associated with SPT reactivity [45]. In line with this, the recent anthelmintic trial conducted among Vietnamese children [34] found that SPT reactivity was inversely associated with higher IL-10 in response to hookworm antigen [34] and that after 12 months of deworming, there was a trend towards lower IL-10 responses in the treatment group although this was not statistically significant [34]. In contrast however, a study among Ecuadorian children living in a helminth-endemic area, observed no relationship between *A. lumbricoides* antigen induced IL-10 (or the frequency of IL10+ T cells) and SPT reactivity [46].

In another study in Ecuador, at the end of 12 months of deworming with albendazole (cluster-randomized study design), Cooper and colleagues examined whole blood cytokine responses of 214 children from 42 schools selected from a total of 1,632 children [47]. Results indicated that anthelmintic treatment was associated with enhanced Th2 cytokine responses to *A. lumbricoides* adult worm antigen (but lower IL-10 responses) as determined by whole blood cultures. Although this profile would support the notion that allergic responses increase with deworming, the investigators did not see differences in these cytokines in SPT positive versus negative subjects [47]. In addition, as one of the few studies using cytokine responses to aeroallergens (*Dermatophagoides pteronyssinus* and *Periplaneta americana*) the group found no differences between treated and untreated subjects. However, like studies conducted in affluent countries [48], the induction of cytokine production by these allergens was altogether very low.

Regarding the development of Th2 immune responses from infancy, a birth cohort study performed in a helminth-endemic area near Jakarta, Indonesia followed Th2 immune responses from 2 to 48 months among 240 children whose mothers were

recruited during pregnancy [49]. In this study, whole blood cultures were used to assess Th2 cytokine responses by measuring IL-5 response to a mitogen as well as to helminth antigens at 5 time-points between 2 and 48 months. The study found that substantial Th2 responses were seen from 5 months onwards and increased over time. Concurrently, total IgE levels were shown to gradually increase over time peaking at 48 months. Remarkably, when SPT reactivity was assessed at 48 months, strong Th2 immune responses did not translate into SPT reactivity [49]. Rather, low maternal education was associated with reduced odds of SPT reactivity while maternal infection with the filarial worm *Wuchereria bancrofti* during pregnancy tended to reduce the odds of SPT reactivity but this was not statistically significant. This longitudinal study demonstrates how children born into helminth-endemic areas develop strong Th2 responses that increase with age but do not translate into allergic response.

A number of recent reports from the SCAALA cohort of urban children aged 4-11 living in Salvador, Brazil where past and current infections (helminth, viral and bacterial) were associated with reduced SPT reactivity, have provided further insight into the immunological control of allergies in an emerging economy [50-53]. First, the effect of environmental exposures on cytokine production in unstimulated whole blood from 1376 children was examined [50]. It was observed that the proportion of children spontaneously producing IL-10 was significantly greater among those without access to drinking water [50]. It was also found that intestinal helminth infection was associated with the induction of immune hyporesponsiveness that was stronger in children producing spontaneous IL-10 [51]. Later, cytokine responses from whole blood cultures stimulated with mitogen were measured in 1127 children and different immunological phenotypes were defined: 'responsive' (characterized by generalized cytokine production above cytokine detection limits), 'under-responsive' (characterized by few responses above the detection limit) and 'intermediate' [52]. The responsive phenotype was strongly associated with higher maternal education, adequate street paving and light infection burden. Furthermore, the responsive phenotype was also linked to increased odds of SPT reactivity as well as allergen-specific IgE sensitization. Thus, enhanced immune responsiveness seemed to be linked to environmental factors and atopic outcomes. However, the study found no evidence of a significant association between the different immune phenotypes and reported wheezing or asthma. This is consistent with other epidemiological study findings where effects are observed for allergic sensitization but less for wheeze or asthma [54].

Aside from immune profiles based on cytokine responses, other mechanisms have also been investigated [55-57]. One of particular interest is a study determining whether basophil suppression occurs in humans infected with helminths. Larson *et al.* examined histamine release by whole blood cells of a subset of 28 helminth-infected children from Ecuador aged 8-14 years before and two weeks after anthelmintic treatment [58]. Stimulation of blood with anti-IgE showed a considerable increase in basophil activation post helminth treatment. This indicates that the ability of basophils

to respond to both IgE-dependent and IgE-independent activation is suppressed during intestinal helminth infection in humans [58]. Given the role of basophils as effector cells in the allergic immune response, suppression of basophil functionality may be an additional mechanism by which helminths protect against allergies [58].

Helminth-induced IgE cross-reactivity

Cross-reactivity reflects the phylogenetic relations between organisms that results in a large degree of homology in the primary structure of proteins eventually leading to homologous three dimensional structures and potential cross-reactivity [59]. Since the 1980s, two types of IgE cross-reactivity related to allergy have been recognized: cross-reactivity due to proteins and cross-reactivity due to glycans on glycoproteins known as cross-reactive carbohydrate determinants (CCDs) [59].

The first indications of possible helminth involvement in IgE cross-reactivity came from observations in population studies of elevated levels of allergen specific IgE without skin reactivity or symptoms among helminth-infected children [3].

A. Protein cross-reactivity and helminths

Although there has been extensive characterization of cross-reactivity between plant-derived proteins for example between birch and apple allergens, cross-reactivity between allergens from invertebrates such as mites, shrimp, cockroach and schistosomes is a growing area of interest [59]. A number of proteins such as tropomyosin [60], paramyosin [61] and glutathione-S-transferase (GST) [62] have recently been studied in some detail. In Brazil, among children aged 3 to 6 years from a helminth-endemic area as well as patients with cockroach allergy from an allergy clinic, a strong correlation was observed between IgE against *Ascaris* tropomyosin and IgE against *P. americana* tropomyosin [60]. Notably, 75.6% of the children from the helminth-endemic area had IgE antibodies against cockroach tropomyosin yet had no symptoms of cockroach allergy [60].

IgE cross-reactivity between house dust mite tropomyosin (Der p 10) and the filarial nematode *Onchocerca volvulus* has also been demonstrated and may account for elevated levels of mite-specific IgE seen in helminth exposed individuals [63]. With respect to GST protein, GST from cockroach (Bla g 5) and from the filarial worm *W. bancrofti* (WbGST) were found to be 30% identical at the amino acid level and IgE against Blag 5 strongly correlated with IgE against WbGST [62].

An analysis of cross-reactivity between extracts of *A. lumbricoides* and dust mite allergens was conducted in the Philippines in subjects with high levels of IgE to extracts of *Ascaris* and mite allergens [61]. Absorption assays demonstrated that *A. lumbricoides* antigens could inhibit up to 92% of mite-specific IgE among allergic subjects while mite allergens inhibited up to 54% of *Ascaris*-sIgE among *Ascaris*-infected subjects. IgE responses to the recombinant form of the paramyosin *Blomia* allergen (Blo t 11)

were also assessed and positive rBlot t 11-fD –specific IgE reactivity was seen in 80% of allergic subjects and 46% of *A. lumbricoides* positive subjects thus indicating cross-reactivity between paramyosin from *Blomia* and paramyosin from *A. lumbricoides* [61].

In view of helminth-associated IgE cross-reactivity, Carvalho and colleagues evaluated the use of IgE responses to recombinant *Blomia* allergens (rBlo t 5 and rBlo t 21) to improve specificity in determining allergy to mite in a population from the tropics [64]. To this end, sera from a subset of children (N=35) enrolled in the SCAALA study in Brazil all of whom had elevated allergen-specific IgE was assessed for IgE reactivity to recombinant *Blomia* allergens. The study found that 82.9% of the children who had elevated IgE against *B. tropicalis* extract had IgE to rBlo t 5 and rBlo t 21 [64]. Absorption assay results showed that pre-incubation with *Ascaris* antigens affected IgE reactivity to *B. tropicalis* extract but not to rBlo t 5 and to rBlo t 21. This study demonstrates the value of using recombinant mite allergens rather than crude mite extract for serodiagnostic purposes in a population from a helminth-endemic area.

B. Carbohydrate cross-reactivity and helminths

One of the earliest investigations into carbohydrate cross-reactivity observed that one third of grass pollen-sensitized individuals in an outpatient population in the Netherlands had elevated levels of IgE against peanut extract without peanut SPT reactivity or peanut allergy symptoms [65]. Further analysis revealed that in 91% of cases, IgE directed against N-linked carbohydrates of glycoproteins known as cross-reactive carbohydrate determinants (CCDs) could be detected [65]. Cross-reactive IgE directed against CCDs in this population was also demonstrated to have poor biological activity [65]. It is estimated that between 15% and 30% of allergic patients generate IgE directed against glycans [66]. The two major N-glycan motifs involved in cross-reactivity are xylose and core-3-linked fucose which are found in plants and invertebrates including helminths [67].

A prominent feature of helminth infections is elevated levels of IgE directed against allergens without SPT reactivity [68]. A recent study in Africa has demonstrated that carbohydrate cross-reactivity might play a role in this phenomenon [69]. In schoolchildren aged 5-16 years in Ghana, 17.5% of subjects were IgE sensitized to peanut (≥0.35 kU/L) yet 92.4% of those sensitized were peanut SPT negative [69]. In addition, current infection with *S. haematobium* was strongly associated with peanut IgE sensitization and a strong correlation was observed between IgE against whole peanut extract and IgE against CCDs. Notably, inhibition assays demonstrated that not only could this IgE against whole peanut extract be almost completely inhibited by the CCD marker bromelain, but also by *S. haematobium* soluble egg antigen which is enriched with N-glycans [69]. Furthermore, basophil histamine release assays demonstrated that the IgE directed against peanut in this population had low biological activity [69]. This study provides a model which proposes that in helminth infections, primary sensitization may occur to carbohydrate moieties present in helminths which

are also present in some well-characterized allergens such as peanut and that such IgE antibodies have low biological activity (Figure 3).

Although the lack of clinical relevance of IgE antibodies against CCDs has been demonstrated [70], in recent years, IgE directed against the carbohydrate epitope galactose-α-1,3-galactose (α-gal) has been linked to two forms of anaphylaxis [71]. The first being immediate onset anaphylaxis following the first infusion of the monoclonal antibody cetuximab among patients from the Southeastern United States receiving therapy for cancer [72]. Further analysis determined that reactions occurred in patients with pre-existing IgE against α-gal [71] possibly induced by the lone star tick *Amblyomma americanum* [73]. The second form of anaphylaxis associated with α-gal is delayed onset reactions 3-6 hours following ingestion of mammalian meat products (Figure 3) [71]. Although the reasons behind the delay in the onset of reactions are

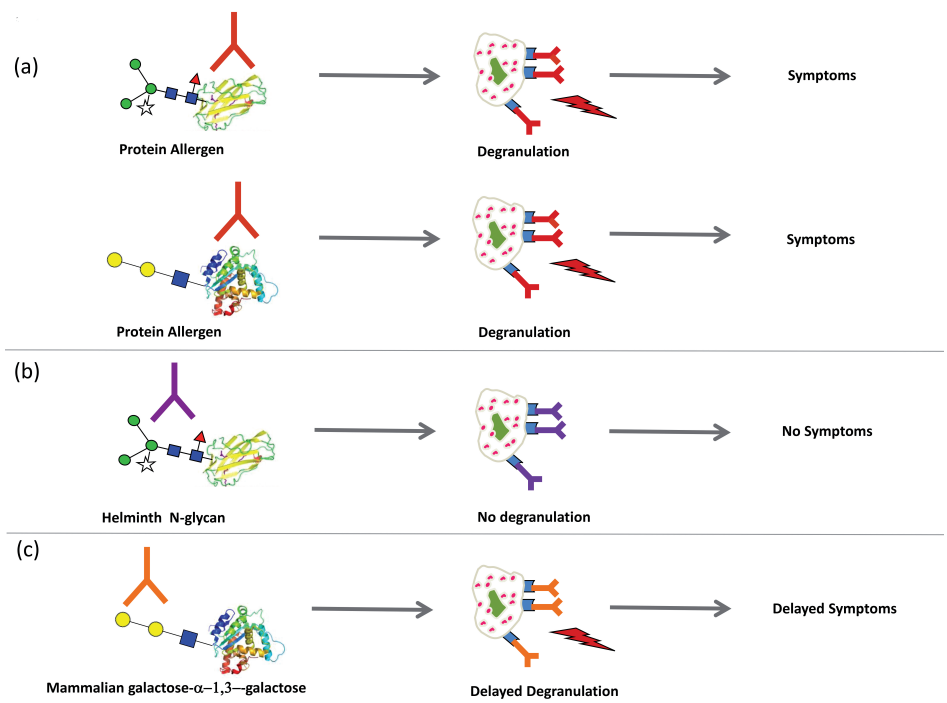

Figure 3: Immunoglobulin E antibody binding to different allergen epitopes

A simplified illustration of IgE antibody binding to different allergen epitopes. (a) IgE directed against protein epitopes of an allergen has strong biological activity and can lead to mast cell degranulation and allergy-related symptoms. (b) IgE directed against the N-glycan xylose or core-3-linked fucose carbohydrate epitopes has low biological activity and does not result in strong mast cell degranulation and related symptoms (c) IgE directed against the galactose-α-1,3-galactose (α-gal) carbohydrate epitope can result in mast cell degranulation and delayed symptoms. However, in some cases where α-gal is intravenously infused, symptoms can be immediate. The different colours of IgE antibodies are used to suggest strong (red), weak (purple) or intermediate (orange) biological activity.

yet to be fully understand, it is believed that it represents the time for red meat to be digested and for associated lipids to be absorbed [71].

Interestingly, in serum samples from children living in rural helminth-endemic communities in Kenya and Ecuador, positive IgE responses to α-gal have been observed which could be tick-related but could also indicate the involvement of helminths or other ectoparasites [71]. In addition, a study conducted in Zimbabwe examined IgE responses against α-gal in rural helminth-infected subjects as well as urban doctor-diagnosed cat allergic patients [74]. In the parasite-infected group, 85% had IgE against α-gal and 66% had IgE against the cat allergen Fel d 5 found in cat dander extract (CDE) [74]. The IgE to α-gal and IgE to Fel d 5 were highly correlated which is in line with recent studies that have demonstrated that α-gal is present on Fel d 5 [75]. Furthermore, only 2 out of 47 of the parasite-infected had IgE to the recombinant form of the cat allergen Fel d 1 which does not carry α-gal. By contrast, among the cat allergic patients, only a few had IgE responses to Fel d 5 and α-gal while 74% had responses to recombinant Fel d 1 [74]. These observations imply that in helminth-endemic areas, the IgE to α-gal is not clinically relevant. However, given that no information was collected on reactions to mammalian meat in the helminth-endemic areas [76], more in-depth studies are needed to assess the prevalence of sensitization to α-gal in populations in different geographical areas and the relationship between sensitization and clinical outcomes.

Future directions

Recombinant allergen technology

IgE cross-reactivity between helminth antigens and environmental as well as food allergen extracts has demonstrated the potential limits in diagnostic value of testing IgE responses against whole allergen extracts in helminth-endemic populations. Therefore, the use of recombinant allergen technology for the evaluation of IgE responses to allergens is much needed for better specificity and to improve diagnostic accuracy.

Helminth products as therapies

Given the abundant evidence from epidemiological and experimental studies of the immunomodulatory properties of helminths, in the past few years, steps have been taken towards harnessing the potential of helminths and their products in the treatment of allergies as well as autoimmune conditions. One such therapeutic possibility is the use of eggs from the pig nematode *Trichuris suis* which was first used in clinical trials to treat inflammatory bowel disease [77]. Since then, a double-blind, placebo-controlled, parallel group trial among adults in Denmark has examined the efficacy of *T. suis* ova therapy in the treatment of grass pollen-induced allergic rhinitis and has shown no therapeutic effect [78]. There are currently 13 active or completed clinical trials with *T. suis* eggs [79] and their results will establish whether treatment later in life (as opposed to in early life) with relatively low exposure to helminths might be effective.

In addition, hookworm larvae have also been used to infect human volunteers with the view towards a potential therapy for inflammatory diseases [80]. For helminthic therapy research in general, much effort is being put into the characterization of helminth-derived molecules with modulatory activity to be able to treat patients with well-defined products rather than full infections [81].

Novel allergens relevant in the tropics

The recent study on peanut allergy among schoolchildren in Ghana found that in a subset of those with elevated IgE responses to whole peanut extract, a few had elevated IgE to the recombinant form of the peanut allergen Ara h 9 [69]. Furthermore, IgE antibodies against Ara h 9 were biologically active at low allergen concentrations as determined by basophil histamine assays. Ara h 9 is a member of the nonspecific lipid transfer protein (LTP) family of allergens and appears to play a role in peanut allergy among patients in the Mediterranean region [82]. It is believed that the peach LTP allergen Pru p 3 may act as primary sensitizer among peanut allergic subjects in Spain [83]. The origin of sensitization to LTPs in areas of the tropics such as Ghana and the role of helminths remain unknown but provide future directions for further research. Aside from the case of Ara h 9, other novel allergens found in the tropics exist that require better characterization [84].

Concluding remarks

The intersection between helminths and allergies has been an interesting area of research which has shed light on immunological pathways, on allergen structure and on cross-reactivity as well as on differences in allergic phenotypes / outcomes in different geographical locations. Future studies have to build on these findings in order to generate the tools to diagnose, treat and prevent allergic disorders not only in affluent countries where they are rampant but also in areas where allergies are emerging as chronic diseases of public health importance.

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Background Information

chapter 8

Summarizing Discussion

Introduction

There is overwhelming evidence that reduced exposure to microorganisms or parasites during childhood may lead to the inadequate maturation of the regulatory component of the developing immune system. This immune dysregulation, which has been closely linked to lifestyle changes and urbanization, is thought to be one of the key factors driving the global rise in inflammatory conditions that include allergies. Helminth infections, which have strong effects on the immune system, are one of the exposures that have changed with lifestyle and urbanization. Indeed, these infections show little geographical overlap with allergic disorders and high burdens of helminth infections are often found in rural areas of developing countries where allergies are least common.

In this thesis, the complex dynamics between helminth infections and allergies among children in Ghana has been examined. Findings include insights into the relationship between helminth infections and allergies as well as urban-rural differences in allergy outcomes. In addition, the thesis explored the role of helminth-induced immunoglobulin E (IgE) cross-reactivity in explaining the lack of skin reactivity to allergens in the face of elevated IgE and also the underlying cellular immune mechanisms related to allergy.

Associations between helminths and allergies

In Chapter 2, the associations between helminth infections and allergy outcomes were examined. Helminth infections were seen predominantly among rural study participants and we observed that schistosome infection was inversely associated with house dust mite skin prick test (SPT) reactivity. Recent cross-sectional studies from helminth-endemic areas support our findings. One investigation conducted among urban children of low socioeconomic status (SES) living in Salvador, Brazil found that heavy infection with the soil- transmitted helminth (STH) *Trichuris trichiura* in early childhood was associated with reduced odds of SPT reactivity later in childhood [1]. Apart from demonstrating the importance of the timing of exposure to helminths, this study also showed that heavy helminth infections (compared to light ones) may have a protective effect against allergies. Similarly, an investigation from South Africa comparing allergy outcomes between rural children and urban low SES children of the same ethnicity, observed that current *Ascaris lumbricoides* infection was associated with reduced odds of SPT reactivity [2].

However, as shown in Chapter 2, the relationship is more complex as we found that although schistosome infection was protective, no association was observed between any of the STHs (*A. lumbricoides*, *Trichuris trichiura* or hookworm) and house dust mite SPT reactivity. The lack of an STH effect may in part be explained by the fact that these infections were not highly prevalent in our study areas. An investigation among Cuban children living in helminth-endemic areas had similar findings to our own in that current intestinal helminth infection was not associated with SPT reactivity [3]. In the Cuban study,

the prevalence of STH infections ranged from 6.3% to 10.6% [3] while ours ranged from 1.9% to 9.9%. Thus, results in Chapter 2 illustrate the importance of taking into account helminth species and degree of endemicity when investigating associations with allergy.

Chapter 2 also assessed whether helminth infections were associated with reported symptoms of allergy. We found that current helminth infection was not associated with reported wheeze or asthma. The effect of helminths on clinical allergy outcomes has been investigated in a number of studies and conflicting findings have been reported. For example, among Afro-Ecuadorian children, heavy *T. trichiura* infection was inversely associated with atopic wheeze [4], while a study in South African children observed that current *A. lumbricoides* infection was associated with increased odds of the asthma marker exercise-induced bronchoconstriction (EIB) [2]. However, in the same South African study, *A. lumbricoides* infection appeared to be associated with a decreased risk of a positive SPT to any aeroallergen [2]. In fact, research findings suggest that in areas with a heavy burden of *A. lumbricoides* infection, this helminth may induce an inflammatory response in the lungs that is independent of the parasite's effect on SPT reactivity [2]. Aside from inflammation associated with the lungs, current *A. lumbricoides* has been linked to a 4-fold reduction in the odds of atopic eczema in rural Cuban children [5]. Hence, it is difficult to conclude whether helminth infections also affect the expression of clinical allergy. Considering that clinical symptoms are relatively rare, it is possible that studies thus far have not been sufficiently powered. Therefore, much larger population studies are needed.

A number of studies have shown that infections other than helminths may play an important role in allergy outcomes. For example, an investigation among urban low SES children in Salvador, Brazil observed that in addition to the protective effect of *A. lumbricoides* infection, past exposure to *Toxoplasma gondii*, Epstein - Barr virus and herpes simplex virus (assessed by seropositivity) were each associated with a lower prevalence of SPT reactivity [6]. These findings highlight the importance of diverse childhood pathogens in reducing the risk of SPT reactivity. Furthermore, among a birth cohort of children from Ethiopia, *Helicobacter pylori* infection determined at 3 years was linked to borderline reduced odds of reported eczema and SPT reactivity to house dust mite [7].

Altogether, there is strong evidence for the protective effects of helminths on allergy outcomes in animal models [8] but the results of cross-sectional studies in humans vary greatly. Though it is generally agreed that helminth infections are often negatively associated with SPT, a lack of association or even positive associations have been observed with lung function. It is also essential to bear in mind that species of helminth as well as timing and burden of infection can all contribute to inconsistent findings in population studies particularly when the study outcome is as complex and multifactorial as clinical allergy. It is also imperative to consider that exposures other than helminths can play an important role in protection against allergy outcomes.

Urban versus rural comparisons and allergy

Part of Chapter 2 examined the urban-rural differences in aeroallergy outcomes and the role of parasitic infections as well as other factors in explaining these differences. In our study population, the most common allergen associated with SPT reactivity was house dust mite and this was most prevalent among urban high SES children (16.3%) followed by rural children (12.1%) and lastly urban low SES children (10.5%).

In other studies performed in central Ghana, the prevalence of SPT reactivity to any allergen was highest in a group of children attending an affluent urban school compared to their less affluent urban counterparts as well as rural children [9, 10]. Whereas the observed gradient in our study was urban high SES > rural > urban low SES, in central Ghana it was urban high SES > urban low SES > rural. Our findings indicate that rural children living in areas endemic for helminths are not always the most protected. In addition, our observations of lower SPT reactivity among urban low SES children who were not highly infected with helminths compared to our rural children, shows that protective factors aside from helminths that are present in urban low SES environments exist and have to be identified.

Another notable finding highlighted in Chapter 2 was that being overweight according to body mass index (BMI) was highest in the urban high SES category followed by rural and lastly, the urban low SES category. Furthermore, a strong association was observed between being overweight and SPT reactivity to house dust mite. These results appear to indicate that despite living in areas endemic for helminths and malaria, rural children in our study may have been of a better nutritional state, as measured by BMI, compared to their urban low SES counterparts. Therefore, rural children may have been more susceptible to allergic reactivity compared to urban low SES children. A relationship between BMI and allergy outcomes has been observed in some population studies. For example, among urban and rural South African children, increasing BMI was significantly associated with EIB as well as a greater strength of association between mite-specific IgE and SPT reactivity to house dust mite [11]. A subsequent investigation also from South Africa found that the consumption of an 'urban diet' partly explains the difference in the prevalence of SPT reactivity to allergens between urban and rural areas [12]. However, the relationship between urban diet and BMI specifically were not examined in this study.

Altogether, there is accumulating evidence to support the fact that determinants associated with lifestyle change such as increasing BMI are linked to allergy outcomes. Results outlined in Chapter 2 also illustrate the problem in labelling areas as rural or urban in rapidly developing countries since in some so-called rural areas, the living conditions and lifestyle may be transitioning to be more pro-allergic than expected. Consequently, there is a critical need for standardized definitions of what constitutes 'rural' and 'urban' environments in rapidly developing countries [13].

In Chapter 3 we reported the results of an in-depth analysis of markers related to food allergy in our study population and whether urban-rural differences existed. From

this cross-sectional study, we observed that the prevalence of SPT to food allergens was similar in urban and rural children but the proportion of rural children reporting adverse reactions was greater than among their urban counterparts which may in part reflect adverse reactions not related to allergy. Chapter 3 also described a nested matched case-control study in which cases were SPT positive for any food allergen and matched controls were SPT negative. A notable finding of this study was that the strength of the association between food-specific IgE and corresponding SPT was greater in the urban compared to rural area. This further demonstrates how environmental factors can modulate the link between IgE and SPT and possibly also the link to reported symptoms.

Helminth-induced IgE cross-reactivity

Although it is clear that different environmental factors play a role in the development of allergic disorders, there is evidence that the presence of certain helminth infections is an important factor associated with lower SPT to allergens. Therefore, a more in-depth understanding of mechanisms behind this association is imperative. In Chapter 4, the effect of helminth-induced IgE cross-reactivity on allergen-specific IgE in our study population was examined. Cross-reactivity is a reflection of the phylogenetic relationship between organisms that leads to a large degree of homology in the primary as well as three dimensional structures of glycoproteins [14]. Therefore, IgE directed against one allergen may recognize homologous structures from other sources. Two types of IgE cross-reactivity related to allergy have been recognized: cross-reactivity due to proteins and cross-reactivity due to glycans on glycoproteins known as cross-reactive carbohydrate determinants (CCDs) [14]. The first indication of possible helminth involvement in IgE cross-reactivity came from observations in population studies where elevated levels of allergen-specific IgE did not translate into skin reactivity or symptoms of allergy among helminth-infected children [15].

As described in Chapter 4, 17.5% of subjects in our study population were IgE sensitized to peanut (≥0.35 kU/L) yet 92.4% of those sensitized were peanut SPT negative. In addition, current infection with *S. haematobium* was strongly associated with peanut IgE sensitization and a strong correlation was observed between IgE against whole peanut extract and IgE against CCDs. Inhibition assays demonstrated that not only could this IgE against whole peanut extract be almost completely inhibited by the CCD marker bromelain, but also by *S. haematobium* soluble egg antigen which is enriched with N-glycans. Moreover, basophil histamine release assays showed that the IgE directed against peanut in this population had low biological activity. Findings in Chapter 4 provide a model which proposes that in helminth infections, primary sensitization may occur to carbohydrate moieties present in helminths which are also present in some well-characterized allergens such as peanut and that such IgE antibodies have low biological activity. Although the lack of clinical relevance of IgE antibodies against CCDs has been demonstrated in Europeans [16], in recent years, IgE directed against the carbohydrate

epitope galactose-α-1,3-galactose (α-gal) has been linked to two forms of anaphylaxis in the Southeastern United States [17]. Interestingly, in serum samples from children living in rural helminth-endemic communities in Kenya, Ecuador and Zimbabwe, positive IgE responses to α-gal have been observed [17, 18]. However, the clinical relevance of IgE to α-gal in helminth-endemic area is yet to be fully established.

Immune mechanisms

In Chapter 5, we focused on underlying immune mechanisms by examining the relationship between cellular immune responsiveness and SPT reactivity to house dust mite. In this chapter, *in vitro* whole blood culture cytokine responses to a panel of stimuli were used to assess general innate and adaptive immune responsiveness. We observed that higher innate as well as adaptive immune responses were associated with being a house dust SPT positive case. Similar observations were made among a cohort of urban low SES children living in Salvador, Brazil where past and current infections (helminth, viral and bacterial) were linked to reduced SPT reactivity [6]. In an immunological study in this Brazilian cohort, cytokine responses from whole blood cultures stimulated with mitogen were measured in 1127 children and different immunological phenotypes were defined: 'responsive' (characterized by generalized cytokine production above cytokine detection limits), 'under-responsive' (characterized by few responses above the detection limit) and 'intermediate' [19]. The responsive phenotype was associated with increased odds of SPT reactivity as well as allergen-specific IgE sensitization. Together, these studies indicate that overall immune hyperresponsiveness may be a characteristic of populations that are at increased risk of developing allergies.

In Chapter 5 it was also noted that this hyperresponsiveness extended to interleukin (IL)-10 production. In other words, high IL-10 in response to innate and adaptive stimulation was associated with increased SPT reactivity. These findings might have been unexpected as a number of investigations in humans have provided evidence that IL-10 plays a key role in the helminth-induced immune regulation of allergic responses [8]. For example, a study in Gabon, determined that IL-10 production by parasite-antigen stimulated peripheral blood mononuclear cells was higher in children infected with *S. haematobium* and elevated IL-10 levels were negatively associated with SPT reactivity to house dust mite [20]. In line with this, an anthelmintic trial conducted among Vietnamese children found that SPT reactivity was inversely associated with higher IL-10 in response to hookworm antigen and that after 12 months of deworming, there was a lower IL-10 response in the treated group although this was not statistically significant [21]. However, a study among Ecuadorian children living in a helminth-endemic area, observed no relationship between either *A. lumbricoides* antigen induced IL-10 determined in whole blood cultures or the frequency of IL10+ T cells and SPT reactivity [22].

Taken together, cellular immunological associations with allergy might be at two different levels. Firstly, general responsiveness and secondly, the type of response.

Future studies need to dissect this by examining responses at the single cell level to understand the sources of different cytokines and to determine the responsiveness of different cell types in allergic and non-allergics.

In Chapter 6, the examination of immune mechanisms was approached by investigating whether the dramatic environmental changes associated with urbanization were having an impact on the developing immune profile at the level of gene expression. The investigation described in Chapter 6 was performed in a subset of study participants and we observed that there were distinct differences in gene expression profiles of children attending schools in rural, urban low SES and urban high SES areas within one geographical region of Ghana. Specifically, higher gene expression levels of IgE, IL-10 and PD-1 were seen in rural compared to urban study participants. Although current *S. haematobium* infection could account for elevated IgE messenger RNA (mRNA) in the rural area, current helminth infection was not associated with elevated IL-10 and PD-1 mRNA in whole blood.

Given the urban-rural difference in IL-10 gene expression, we addressed whether there is a role for IL-10 genetic polymorphisms and found that underlying genetics could not explain the urban-rural difference in IL-10 mRNA. Therefore, high IL-10 mRNA in the rural area may have been a result of either undetected/past helminth infections or other chronic infections and factors. In addition, post-transcriptional regulation of the IL-10 gene has been reported [23] and therefore, IL-10 gene expression may be different from the production of the protein.

As reported in Chapter 6, significant differences in the expression of genes associated with pattern recognition receptor signalling were observed between the two urban schools. Elevated expression of these genes was seen among urban high SES children compared to their urban low SES counterparts. As underlying variations in genetic polymorphisms did not explain observed differences between the two urban groups when it came to TLR-2 and TLR-4, these findings show how even within an urban setting, lifestyle affects gene expression patterns associated with the recognition of microbial products. Thus, Chapter 6 appears to support studies that emphasize the influence of environmental exposures on inflammatory disorders. In fact, the notion that diverse microbial exposures in childhood may protect against the development of allergies and other inflammatory disorders has formed the basis for the 'biodiversity hypothesis'. According to this hypothesis, reduced contact with natural environmental microbes as a result of urbanization and lifestyle change may affect the immune-modulatory capacity of human commensal microbiota thus leading to more inflammatory conditions [24, 25].

Helminth and allergies: insights from population studies

Chapter 7 provided a review of the recent literature on helminth infections and allergies from observational and intervention studies. The conflicting findings in the

recent literature were discussed extensively in this chapter. Chapter 7 also covered research areas beyond the scope of this thesis but which are relevant to understanding the relationship between helminths and allergies in childhood.

Limitations and future directions

Limitations of the Study Design

One of the major limitations of the overall investigation described in this thesis is the cross-sectional study design. Cross-sectional studies examining associations between helminths and allergy outcomes are prone to the problem of temporality [26] since all parameters of interest are determined at the same time. Therefore, prospective studies are needed to fully investigate causality. Another important future direction would be to examine the effect of treatment with anthelmintics on allergy outcomes among Ghanaian children. In addition, the studies described in this thesis focused on allergy outcomes in children aged 5-16 years. It is imperative that future investigations in Ghana include other age-groups such as children under 5 years as well as adults.

Markers of urbanization and lifestyle change

As more investigations in developing countries such as Ghana examine the effects of urbanization on health outcomes, better characterization of urban and rural areas based on standardized indicators are needed. In addition, standardized markers of individual socioeconomic status are imperative to properly investigate the impact of lifestyle change on health outcomes.

Molecular diagnostics in helminth detection

A limitation of the study described in this thesis was that the diagnosis of helminth infections was based on a single sample. This may have led to an underestimation of the helminth burden in our study population. Therefore, future investigations would not only have to incorporate the collection of multiple samples but also molecular diagnostic techniques such as real-time PCR [27] and circulating antigen tests to detect schistosome infection [28].

Recombinant allergen technology

IgE cross-reactivity between helminth antigens and allergens clearly demonstrates the limitations associated with measuring IgE responses against whole allergen extracts in helminth-endemic populations. In recent years, *in vitro* allergy diagnostics in industrialized countries has moved towards component-resolved diagnosis in which purified natural or recombinant allergens are used to detect IgE sensitization to individual allergen molecules [29]. The use of molecular techniques and recombinant DNA technology has allowed the sequencing, synthesizing and cloning of allergenic proteins leading to the production of recombinant allergens for component-resolved

diagnosis [30]. Recombinant allergen technology for the evaluation of IgE responses to allergens in helminth-endemic populations is much needed for better specificity and to improve diagnostic accuracy.

Novel allergens relevant in the tropics

A notable finding highlighted in Chapter 5 was that in a subset of our study participants with elevated IgE responses to whole peanut extract, a few had elevated IgE to the recombinant form of the peanut allergen Ara h 9. Furthermore, IgE antibodies against Ara h 9 were biologically active at low allergen concentrations as determined by basophil histamine assays. Ara h 9 is a member of the nonspecific lipid transfer protein (LTP) family of allergens and appears to play a role in peanut allergy among patients in the Mediterranean region [31]. It is also believed that the peach LTP allergen Pru p 3 may act as primary sensitizer among peanut allergic subjects in Spain [32]. The origin of sensitization to LTPs in areas of the tropics such as Ghana and the role of helminths remain unknown but provide future directions for further research. Aside from the case of Ara h 9, other novel allergens found in the tropics exist that require better characterization [33] including the mammalian carbohydrate α-gal. More in-depth studies are needed to assess the prevalence of sensitization to α-gal in populations in different geographical areas and the relationship between sensitization and clinical outcomes.

Concluding Remarks

The intersection between helminths and allergies in Ghana is an interesting area of research which has shed light on immune responsiveness, on cross-reactivity as well as on variations in allergy phenotypes / outcomes in different geographical locations within one region of Ghana. Future studies have to build on these findings in order to generate the tools to diagnose, treat and prevent allergic disorders in developing countries such as Ghana where allergies are emerging as chronic diseases of public health importance.

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Appendix

Study Questionnaire

GLOFAL
Questionnaire for Allergic Diseases
Multi-Centre Study

Date : __ __/ __ __/ __ __ __ __ Country: *GHANA*
Name of Interviewer : __
Name of Recorder : __

A. Child's details

1. Name / ID number :__ __ __ __ __ __ __ __ __ __ __ __ __ __/ __ __ __ __ __
2. Date of birth / Age : __ __/ __ __/ __ __ __ __ [ ] year(s)
3. Place of Birth (including Region): __ __ __ __ __ __ __ __ __ __ __ __ __ _____
   [Country IF NOT Ghana]: __ __ __ __ __ __ __ __ __ __ __ __ __ ___
4. Ethnicity: __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ _
   [Country of Origin IF NOT Ghana]: __ __ __ __ __ __ __ __ __ __ __ __ _
5. Sex : [ ] Male [ ] Female
6. What is the position of this child in sib-ship? : __ __ __ __ of __ __ __ children
7. School information
   Class : __ __ __ __ __ __ __ __
   Name of school : __
   __
8. House information
   House Number : __ __ __ __ __ __ __ __
   Suburb/ Area : __ __ __ __ __ __ __ __
   Telephone : __ __ __ __ __ __ __ __

   GPS Readings
   a. Latitude : __ __ __ __ __ __ __ __
   b. Longitude : __ __ __ __ __ __ __ __
   c. Altitude : __ __ __ __ __ __ __ __
9. How far is the school from home (GPS reading)? : __ __ __ __ __ __ __ Km

10. How does the child get to school most of the time?

[ ] Walk
[ ] Bicycle
[ ] Taxi
[ ] Bus / Trotro
[ ] Private Car
[ ] Other, please specify __ __ __ __ __ __ __ __ __ __

B. Socio-economic Status and Environmental Factors.

1. Has the child lived in this town/village since he/she was born? [ ] Yes [ ] No

2. If you answered "no" where has the child lived before and for how long?

Area A In. __ __ __ __ __ __ __ __ __ **for**__ __ __ **month(s)** __ __ __ **Year(s)**
Area B In. __ __ __ __ __ __ __ __ __ **for**__ __ __ **month(s)** __ __ __ **Year(s)**
Area C In. __ __ __ __ __ __ __ __ __ **for**__ __ __ **month(s)** __ __ __ **Year(s)**
Area D In. __ __ __ __ __ __ __ __ __ **for**__ __ __ **month(s)** __ __ __ **Year(s)**

3. Who provides financially for this child?

[ ] Father and Mother
[ ] Father
[ ] Mother
[ ] Other, please specify: __ __ __ __ __ __ __ __ __ __ __ __ __

4. Occupation of person in question number 3: __ __ __ __ __ __ __ __ __ __ __
   Occupation of the spouse of this person: __ __ __ __ __ __ __ __ __ __ __ __ __

5. The highest level of formal education completed by:

Person (question number 3)
[ ] Primary/Elementary [ ] Middle school [ ] JSS [ ] SSS
[ ] O'level [ ] A' level [ ] Vocational/ Commercial
[ ] Training College [ ] Polytechnic/University
[ ] Other, please specify __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

Spouse of this person
[ ] Primary/Elementary [ ] Middle school [ ] JSS [ ] SSS [ ]
O' level [ ] A' level [ ] Vocational/ Commercial
[ ] Training College [ ] Polytechnic/University
[ ] Other, please specify __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

6. Who does this child live with (if different from response in question "3" above)?
[ ] Father and Mother
[ ] Father

[ ] Mother

[ ] Other, please specify: __ __ __ __ __ __ __ __ __ __ __ __ __

7. Occupation of person in question number 6: __ __ __ __ __ __ __ __ __ __ __ __
   Occupation of the spouse of this person: __ __ __ __ __ __ __ __ __ __ __ __ __ __

8. The highest level of formal education completed by:
   Person (question number 6)

[ ] Primary/Elementary [ ] Middle school [ ] JSS [ ] SSS
[ ] O'level [ ] A' level [ ] Vocational/ Commercial
[ ] Training College [ ] Polytechnic/University
[ ] Other, please specify __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

Spouse of this person

[ ] Primary/Elementary [ ] Middle school [ ] JSS [ ] SSS
[ ] O'level [ ] A' level [ ] Vocational/ Commercial
[ ] Training College [ ] Polytechnic/University
[ ] Other, please specify __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

9. The house in which the child lives is made primarily of :

[ ] Cement [ ] Wood [ ] Mud [ ] Other, please specify __ __ __ __ __

10. What is the main source of water supply to the home?

[ ] Pipe-borne [ ] Tanker (treated) [ ] Tanker (untreated)
[ ] River/ Stream [ ] Well/ Borehole
[ ] Other, please specify __ __ __ __ __ __ __ __ __ __ __ __ __

11. What is the type of toilet in the home?

[ ] Indoor WC [ ] Compound latrine [ ] Public latrine
[ ] Other, please specify __ __ __ __ __ __ __

12. The fuel mostly used at home for cooking is*(tick one):*

[ ] LPG [ ] Electricity [ ] Charcoal [ ] Firewood
[ ] Kerosene [ ] Other__ __ __ __ __ __ __

13. What kind of accommodation does the child live in?

[ ] Detached house [ ] Semi-detached [ ] Flat
[ ] Compound house [ ] Other, please specify__ __ __ __ __ __ __ __

14. How much money did your family spend on electricity in the past month?

¢ __ __ __ __ __ __ __ __ __
[ ] Respondent unable to estimate

15. How much money did your family spend on food in the past month?

¢ __ __ __ __ __ __ __ __ __
[ ] Respondent unable to estimate

C. ISAAC Core Questionnaires

C1 Core Questionnaire for Wheezing and Asthma

All questions are about problems which occur when this child <u>DOES NOT</u> have a cold or the flu

1. Has this child ever had wheezing or whistling in the chest?

[ ] Yes **[ ] No**

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

2. Has this child had wheezing or whistling in the chest in the past 12 months?

[ ] Yes **[ ] No**

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

3. How many attacks of wheezing has this child had in the past 12 months?

[ ] None
[ ] 1-3
[ ] 4-12
[ ] > 12

4. In the past 12 months how often, on average, has this child's sleep been disturbed due to wheezing?

[ ] Never woken with wheezing
[ ] Less than one night per week
[ ] One or more nights per week

5. In the past 12 months, has wheezing ever been severe enough to limit this child's speech to only one or two words at a time between breaths?

[ ] Yes **[ ] No**

6. In the past 12 months, has this child's chest sounded wheezy during or after exercise?

[ ] Yes **[ ] No**

7. In the past 12 months, has this child had a dry cough ***at night***, apart from a cough associated with a cold or chest infection?

[ ] Yes **[ ] No**

8. Has a doctor ever diagnosed your child as having asthma?

[ ] Yes **[ ] No**

9. If yes to question number 8, what is the name of the medicine(s) the doctor gave to your child?

Medicine(s) __

[ ] Cannot recall name of medicine

10. Has any member of this child's family ever had asthma?

[ ] Yes [ ]No [ ] No idea

11. If you answered "yes" to question 10, indicate relationship to child (*tick all that apply*)

[ ] Father
[ ] Mother
[ ] Brother or Sister
[ ] Father's __ __ __ __ __ __ __ __ __ (family member eg. sister, father)
[ ] Mother's __ __ __ __ __ __ __ __ __ (family member eg. sister, father)

C2 Core Questionnaire for Rhinitis/Hayfever

All questions are about problems which occur when this child <u>DOES NOT</u> have a cold or the flu.

1. Has this child ever had a problem with sneezing or a runny or blocked nose (nose problem) without cold or the flu?

[ ] Yes [ ] No

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

2. Has this child had this nose problem in the past 12 months?

[ ] Yes [ ] No

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

3. In the past 12 months, has this child's nose problem been associated with itchy-watery eyes?

[ ] Yes [ ] No

4. In which of the past 12 months did this nose problem occur? (please tick any which apply)

[ ] January
[ ] February
[ ] March
[ ] April
[ ] May
[ ] June
[ ] July
[ ] August
[ ] September
[ ] October
[ ] November
[ ] December
[ ] Rainy season
[ ] Dry season
[ ] Anytime
[ ] No idea

5. In the past 12 months, how much did this nose problem interfere with this child's daily activities such as school or play?

[ ] Not at all
[ ] A little
[ ] A Moderate
[ ] A lot

6. Has a doctor ever diagnosed your child as having allergic rhinitis / hay fever?

[ ] Yes [ ] No

7. If "Yes" to question number 6, what is the name of medicine the doctor gave your child?

Medicine(s) __

[ ] Cannot recall name of medicine

8. Has any member of this child's family ever had allergic rhinitis / hay fever?

[ ] Yes [ ] No [ ] No idea

9. If you answered "yes" to question 8, indicate relationship to child (*tick all that apply*)

[ ] Father
[ ] Mother
[ ] Brother or Sister
[ ] Father's __ __ __ __ __ __ __ __ __ (family member eg. sister, father)
[ ] Mother's __ __ __ __ __ __ __ __ __ (family member eg. sister, father)

C3 Core Questionnaire for Atopic Dermatitis/Eczema

Show pictures from the* "Observer's protocol for recording signs of visible flexural dermatitis"** ***to the respondent

1. Has this child ever had one or more skin problem(s) like in the pictures accompanied by an itchy rash which was coming and going for at least 6 months?

[ ] Yes, Picture number__ __ __ __ [ ]No

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

2. Has this child ever had this skin problem (itchy rash) in the last 12 months?

[ ] Yes [ ] No

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6

3. Has this skin problem (itchy rash) at any time affected any of the following places: The folds of this child's elbows, behind the knees, in front of ankles, under the buttocks or around the neck, ears or eyes?

[ ] Yes [ ] No

4. How often, on average, has this child been kept awake at night by this itchy rash?

[ ] Never in the past 12 months
[ ] Less than one night per week
[ ] One or more nights per week

5. Did this rash clear completely at any time during the past 12 months?

[ ] Yes [ ] No

6. Has a doctor ever diagnosed your child as having allergic eczema/ atopic dermatitis?

[ ] Yes **[ ] No**

7. If yes to question number 5, what is the name of the medicine(s) the doctor gave to your child?

Medicine(s) __

[ ] Cannot recall name of medicine

8. Has any member of this child's family ever had allergic eczema/ atopic dermatitis?

[ ]Yes **[ ]No** **[ ]No idea**

9. If you answered "yes" to question 8, indicate relationship to child (*tick all that apply*)

[ ] Father
[ ] Mother
[ ] Brother or Sister
[ ] Father's __ __ __ __ __ __ __ __ __ (family member eg. sister, father)
[ ] Mother's __ __ __ __ __ __ __ __ __ (family member eg. sister, father)

D. Health concerns

1. When was the last time this child had any medical treatment?

[ ] < 1 month ago **[ ] 1 to 3 months ago**
[ ] 3 to 6 months ago **[ ]> 6 months**

2. What was the name of the medicine in '1' and for which condition was it given?

Medicine1__ __ __ __ __ __ __ __ __ __ Condition1 __ __ __ __ __ __ __ __ __
Medicine2__ __ __ __ __ __ __ __ __ __ Condition2 __ __ __ __ __ __ __ __ __
Medicine3__ __ __ __ __ __ __ __ __ __ Condition3 __ __ __ __ __ __ __ __ __

3. When did this child last have treatment for worm infection?

[ ] < 1 month ago **[ ] 1 to 3 months ago**
[ ] 3 to 6 months ago **[ ]> 6 months**
[ ] No idea

4. What was the name of the medicine used when this child was last treated for worm infection? __ __ __ __ __ __ __ __ __ __ __ __ __

[ ] Cannot recall name of medicine

5. Is there any smoker in your house

[ ]Yes **[ ]No**

6. If you answered "yes" to question 5, does this person smoke when the child is present?

[ ]Yes **[ ]No**

7. Is this child exposed to tobacco smoke outside your home?

[ ] Yes [ ] No [ ] No idea

8. Do you use groundnut oil for any other purpose (example as skin ointment)?

[ ]Yes [ ]No

E. Diet

For this section, please ask the respondent how frequently **the child** consumes each food item and the cooking method used to prepare the food item.

FOOD ITEM	FREQUENCY OF CONSUMPTION					COOKING METHOD			
Staples	**Daily**	**1x weekly (at least)**	**1x monthly (at least)**	**Every half year (at least)**	**Never**	**Boiling/ Steaming**	**Frying**	**Smoking/ Grilling/ Roasting**	**Other**
Rice	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Cassava	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Plantain	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Yam	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Maize/ Corn	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Meat/Protein Source									
Cattle (Beef)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Sheep (Mutton)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Goat (Chevron)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Pig (Pork)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Chicken	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Fish	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Other__ __ __ __ __ __ __	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Shellfish (Crab, shrimp etc)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Snail	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Mushroom	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Beans	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Eggs	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Other__ __ __ __ __ __ __	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _

FOOD ITEM	FREQUENCY OF CONSUMPTION					COOKING METHOD			
	Daily	1x weekly (at least)	1x monthly (at least)	Every half year (at least)	Never	Boiling/ Steaming	Frying	Smoking/ Grilling/ Roasting	Other
Industrially processed oils									
Cooking oil (e.g. Frytol, Palmin)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Other__ __ __ __ __ __ __	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Home-made oils									
Palm oil	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Palm Kernel oil	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Coconut Oil	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Groundnut oil	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Shea Butter	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Other__ __ __ __ __ __ __	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Evaporated Milk (eg. Ideal)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Powdered Milk (eg Nido)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Margarine (e.g. Blue Band)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Cheese/Butter (e.g. Even, Laughing Cow)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Ice-cream/ Yoghurt (e.g. Fanice or FanYogo)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Groundnuts/ Peanuts	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Pastries (e.g. Cakes, pies,)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Fresh fruits (e.g., orange)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _
Fresh vegetables (e.g. salads, fresh ground pepper sauce)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	__ __ __ __ _

F. Food Allergy

The following questions are about the child's reactions to food OTHER than reactions caused by food poisoning, illness or a bacterial infection such as cholera etc.

1. Has your child ever had adverse events (reactions) after food intake?

[ ] Yes **[ ] No**

If yes which food? __
__ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __
(Show list in **Table F2** if needed)

2. Has the child had any problems eating any other food or foods that not listed in **Table F2**?

If yes please list:

__ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

__ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

IF YOU HAVE ANSWERED "NO" TO QUESTION 1 & 2 PLEASE SKIP TO QUESTION 13

3. How old was the child when s/he had the first problem eating this food?

__ __ __ __ **year(s)**

4. How old was the child when s/he had the most recent problem eating this food?

__ __ __ __ **year(s)**

5. Has the child had this illness or trouble after eating this food?

[ ] Only once
[ ] 2-4 times
[ ] More than 4 times

6. Has the child avoided eating that food since the illness or trouble?

[ ] Yes **[ ] No**

7. Did this illness or trouble include any of the following? (please mark if yes)

Table F1

	Yes	No
Itching, tingling or swelling in the mouth, lips or throat	[ ]	[ ]
A rash, nettle sting-like rash or itchy skin	[ ]	[ ]
Diarrhoea or vomiting (other than food poisoning)	[ ]	[ ]
Runny or stuffy nose	[ ]	[ ]
Red, sore or running eyes	[ ]	[ ]
Difficulty swallowing	[ ]	[ ]
Breathlessness	[ ]	[ ]
Stiffness in your joints	[ ]	[ ]
Fainting or dizziness	[ ]	[ ]
Headaches	[ ]	[ ]

8. Has the child had any other symptoms?

[ ]Yes [ ]No

If yes, please describe __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

9. How long after eating the food did the child start having the first symptom?

[ ] Minutes [ ] Hours [ ] Days

10. How long did it last?

[ ] Minutes [ ] Hours [ ] Days

11. Did the child receive any treatment?

[ ]Yes [ ]No

12. If yes for question number 11, what was the name of medicine given?

__ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __

13. Have you ever been told by a doctor that the child has a food allergy?

[ ]Yes [ ]No

14. Say approximately how often the child eats the following foods, and whether or not s/he avoids them because they make him/her ill:

Table F2

Food	Any Reaction		How often does the child eat this food (in season) *Tick one column only*					Does the child avoid this food because it makes him/her ill?	
	Yes	No	Most Days	Most weeks	Most months	Rarely	Never	Yes	No
Cow's milk	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Hen's eggs	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Fish	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Shrimp	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Groundnuts	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Pineapple	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Banana	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Apple	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Cassava	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Soybean	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Mango	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Pawpaw	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Plantain	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Coconut	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Wheat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Sweet potato	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Potato	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Sorghum	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Millet	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Carrot	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Avocado	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Beans	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Tomato	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Orange	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Palm Nut	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Corn	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Melon	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Rice	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Water Yam	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Cocoyam	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Kontomire	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Okro	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Flour	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Nutmeg	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

G. Early Life factors

*For the following questions please ask to see the **child's weighing card**.*

Is the child's weighing card available? [ ] Yes [ ] No

1. Was your child born prematurely?
[ ] Yes [ ] No

2. If Yes, how many months premature?
_____ (in months)

3. What was your child's weight at birth?_____ kg
Date Recorded ___/___/___

4. After birth, when did your child START breastfeeding:
[ ] After Hours [ ] After Days [ ] After Weeks

5. For how long was your child breast-fed?
Duration_____ (in months)

6. For how long was your child fed with ONLY breast-milk?
Duration____(in months)

7. What was the first food OTHER than breast-milk given to your child?
Food_____ at what age? _____ (in months)

8. Was your child breast-fed by anyone OTHER than his or her mother at any point?
[ ] Yes [ ] No

9. What were the reasons that your child was stopped breastfeeding?
Please state these reasons:

_____
_____
_____

10. Did your child receive the following immunizations: (**Please Verify with Child's Immunization Record**)

	No	Yes	Not Sure
Oral Polio Vaccine (OPV)			
Bacillus Calmette Guérin (BCG)			
Diphtheria Pertussis Tetanus (DPT)			
Yellow Fever			
Measles			

Other Immunizations, please state: _____
_____

11. In your child's first 2 years of life, were you told by a health worker such as a doctor or nurse that your child suffered from any of the following? :

	No	Yes	Cannot Recall
Respiratory infection such as Pneumonia or Bronchiolitis			
Bacterial Meningitis			
Worm infection			
Measles			
German Measles (Rubella)			
Hepatitis A			

Other Diseases/Infections, please state:

_____

_____

_____

12. In your child's first 2 years of life, did he or she attend a crèche or nursery?

[ ] Yes **[ ] No**

Summary

Helminth Infections and Allergies in Ghana

Over the past few decades, there has been a dramatic rise in the prevalence of allergic disorders worldwide especially among children. This global increase has been linked to improved hygiene, better standards of living and fewer childhood infections. Infections in childhood are thought to be an essential part of the education of a developing immune system. In fact, immune function evolved in pathogen-rich environments to ensure a balance was maintained between strong effector mechanisms that counter pathogens and regulatory mechanisms that modulate these effector responses to prevent excessive inflammation. These regulatory mechanisms have been exploited by some micro-organisms and parasites to their own benefit. For example, chronic infections with parasitic worms known as helminths have been shown to induce such regulatory mechanisms which down-modulate the host's immune system and ensure the worm's own survival. Individuals with chronic helminth infections have also been shown to be less responsive to vaccines, to self-antigens and to harmless antigens that induce allergic reactions known as allergens. Consequently, through their ability to induce immune hypo-responsiveness, chronic helminth infections may protect against allergic disorders and autoimmunity.

The work highlighted in this thesis explores the complex relationship between helminth infections and allergies among children in Ghana, a rapidly urbanizing country where helminths are still prevalent. Recent studies indicate that allergies are on the rise in Ghana but there is little information on the relationship between helminth infections and allergies among Ghanaian schoolchildren. The study described in this thesis was a large cross-sectional investigation of children aged 5 to 16 years attending schools in urban and rural areas of the Greater Accra Region of southern Ghana.

Chapter 1 provided a general introduction to the research topic of the thesis and the overall objectives. A brief description of the study population and study area was also outlined in this chapter.

In Chapter 2, the relationship between current helminth infection and allergy outcomes was examined in detail. Allergy outcomes were specific immunoglobulin E (IgE) antibodies to house dust mite and cockroach allergens, skin prick test reactivity to these same allergens and information on reported wheeze and asthma. We observed that helminth infections were more prevalent among rural compared to urban children and that infection with the waterborne helminth *Schistosoma* was inversely associated with skin prick test reactivity to house dust mite. At the same time, increasing body mass index was positively associated with skin prick test reactivity to house dust mite. No associations were observed between helminth infections and reported symptoms of allergy (current wheeze and asthma). Findings outlined in this chapter suggest that schistosome infection may play a role in protection against mite skin prick test reactivity in our study population.

In Chapter 3, food allergy was examined among Ghanaian children for the first time. Adverse reactions to food determined by questionnaire were analyzed along

with skin prick test reactivity to peanut and six fruits available locally in Ghana. The most reported adverse reaction to food was to beans followed by pineapple and peanut. The most prevalent skin prick test responses were against pineapple and peanut. A case-control study was performed in a subset of those who were skin prick test positive to food allergens (cases) and in controls that were skin prick test negative. For all study participants in this matched case-control study, IgE antibodies to the food allergens that elicited the SPT responses in the cases were measured. Reported adverse reactions to food among cases and matched controls were also assessed. A good association was observed between elevated IgE antibodies against specific food allergens and corresponding SPT responses and this association was stronger among urban compared to rural children. Overall, the study demonstrated the importance of IgE-mediated adverse reactions to food in Ghanaian children and how notable urban-rural differences in the manifestations of food allergy outcomes existed.

The focus of Chapter 4 was on peanut allergy among children in Ghana. This particular allergy was of interest because peanut consumption in Ghana is known to be high but there are few reports of adverse reactions to peanut in this country. For this investigation, the outcomes used to assess peanut allergy were reported adverse reactions to peanut and peanut sensitization based on serum specific IgE levels as well as skin reactivity. Among study participants, elevated levels of IgE antibodies against whole peanut extract were observed but these levels did not translate into skin prick test reactivity or reported symptoms of peanut allergy. In addition, a strong association was seen between being infected with *Schistosoma haematobium* and IgE antibodies against whole peanut extract. Given this relationship, we went on to characterize the nature of peanut-specific IgE antibodies in a subset of study participants. This was done by examining whether antibodies directed against whole peanut extract would also recognize purified (recombinant) peanut allergen components that are associated with peanut allergy in developed countries. In addition, we also investigated whether peanut-specific IgE would recognize bromelain which is used as a marker of cross-reactive carbohydrate determinants (CCDs). CCDs are carbohydrate structures that are shared by allergenic extracts from different sources ranging from plants to insects and can also be found in helminths. We observed that IgE levels to the component peanut allergens were very low but IgE against bromelain was very high. In addition, IgE against whole peanut extract could be inhibited by bromelain as well as by soluble egg antigen from *S. haematobium* and showed low biological activity. The investigation outlined in Chapter 4 demonstrated that IgE against CCDs, which was possibly induced by past or current *S. haematobium* infection, may account for high levels of IgE to peanut seen among Ghanaian children. These IgE antibodies to peanut had poor biological activity which was supported by the fact that we found no evidence of IgE-mediated peanut allergy among our study population.

The association between immune response at the cellular level and allergy was addressed in Chapter 5. In this chapter, skin prick test reactivity to house dust mite was

used as a marker of allergy. Immune responsiveness described in Chapter 5 was based on cytokine responses determined by *in vitro* whole blood culture assays. The study was performed among a subset of children who were skin prick test positive for house dust mite and negative controls. Overall, we observed enhanced innate and adaptive cellular immune responsiveness associated with house dust mite skin prick test reactivity.

In the previous chapters, notable urban-rural differences were observed when it came to helminth infections and allergy outcomes. Therefore, in Chapter 6, we addressed whether there were significant differences in the gene expression profiles of children in our study population that resided in urban and rural areas. In a subset of participants attending a rural school, an urban low socioeconomic status (SES) school and an urban high SES school, whole blood samples were used to measure the expression of genes related to immune activation and regulation. We found significant urban-rural differences in the expression of genes including the one coding for the regulatory cytokine interleukin (IL)-10. Contrary to expectations, current helminth infection did not explain elevated IL-10 gene expression in the rural area. Moreover, we observed that underlying genetic differences did not fully account for urban-rural variations when it came to IL-10 gene expression. We concluded that past helminth infection or other infections may have played a role in elevated IL-10 expression in the rural area. There were also notable gene expression differences between children attending the two urban schools included in this study. Specifically, the expression of genes coding for receptors involved in the recognition of environmental microbes and pathogens was higher among children attending the urban high SES school compared to the urban low SES school. We speculated that specific lifestyle factors may have had a suppressive effect on the expression of genes involved in the recognition of environmental microbes and pathogens in our study population. This chapter highlighted how immune gene expression patterns are strongly influenced by environmental determinants which may explain the effects of urbanization on health outcomes.

Chapter 7 provided a review of the recent literature on helminth infections and allergies in childhood based on observations from population studies. The insights in this review covered topics ranging from associations in population studies, the effect of anthelmintic treatment on allergic responses, IgE cross-reactivity induced by helminths and an examination of immune mechanisms underlying the relationship between helminth infections and allergies.

The main findings of the thesis were summarized and discussed in Chapter 8 and placed within the context of other population studies. Overall, research into the relationship between helminths and allergies in Ghana has provided insights into immune responsiveness, IgE cross-reactivity as well as insights into variations in allergy phenotypes/outcomes in different geographical locations within one region of Ghana. Future studies are needed to build on these findings to generate tools to diagnose, treat and prevent allergic disorders in developing countries such as Ghana where these conditions are emerging as problems of public health importance.

Nederlandse Samenvatting

Worminfecties en allergieën in Ghana

Het aantal mensen met allergische aandoeningen is de laatste paar decennia wereldwijd dramatisch toegenomen, in het bijzonder bij kinderen. Deze wereldwijde toename wordt toegeschreven aan verbeteringen in hygiëne, levensomstandigheden en een afname van infecties in de kindertijd. Er wordt verondersteld dat infecties in de kindertijd essentieel zijn voor de ontwikkeling van het immuunsysteem. Het immuunsysteem is geëvolueerd in een omgeving rijk aan potentiele ziekteverwekkers, waardoor een balans kon ontstaan tussen sterke afweerreacties die ziekteverwekkers bestrijden en tolerantieprocessen die buitensporige reacties van het immuunsysteem tegen onschuldige stoffen moeten voorkomen. Deze tolerantieprocessen worden door sommige micro-organismen en parasieten uitgebuit. Chronische infecties door parasitaire wormen kunnen bijvoorbeeld tolerantieprocessen in werking stellen die het immuunsysteem van de gastheer onderdrukken en daarmee de overleving van de parasiet veiligstellen. Mensen met een chronische worminfectie reageren daardoor minder goed op vaccins, lichaamseigen stoffen (betrokken bij auto-immuunziekten) en allergenen (betrokken bij allergieën). Als gevolg van deze versterkte tolerantieprocessen kunnen chronische worminfecties mogelijk bescherming bieden tegen auto-immuniteit en allergische aandoeningen.

Dit proefschrift onderzoekt de complexe relatie tussen worminfecties en allergieën bij kinderen in Ghana. Ghana is een land dat snel verstedelijkt en waar parasitaire wormen nog veel voorkomen. Recente studies tonen aan dat het aantal allergische aandoeningen toeneemt in Ghana, maar er is weinig bekend over de relatie tussen worminfecties en allergieën bij Ghanese schoolkinderen. De resultaten die in dit proefschrift worden beschreven zijn gebaseerd op een omvangrijk cross-sectioneel onderzoek onder schoolkinderen in de leeftijd van 5 tot 16 jaar, woonachtig in stedelijke en plattelandsgebieden in Groot-Accra in het zuiden van Ghana.

Hoofdstuk 1 bevat een algemene inleiding tot het onderzoeksonderwerp en de algemene doelstellingen van het proefschrift. Daarnaast worden de studiepopulatie en het gebied van de studie beschreven.

In hoofdstuk 2 wordt de relatie tussen worminfecties en allergieën in detail onderzocht. Om te bepalen of er sprake is van allergie hebben we de volgende parameters onderzocht: specifieke immunoglobuline E (IgE) antilichamen tegen huisstofmijt- en kakkerlakallergenen, huidpriktestreactiviteit op dezelfde allergenen en zelf-gerapporteerde gegevens over piepende ademhaling en astma. De resultaten wezen er op dat worminfecties meer voorkomen bij plattelandskinderen dan bij stadskinderen. Ook bleken infecties met *Schistosoma* geassocieerd met lagere huidpriktestreactiviteit op huisstofmijtallergenen. Er werden geen associaties gevonden tussen worminfecties en gerapporteerde symptomen van allergie (piepende ademhaling en astma). De bevindingen van dit hoofdstuk suggereren dat schistosomiasis een beschermende werking kan hebben tegen een positieve huisstofmijt-huidpriktest in onze studiepopulatie.

Voor het eerst is voedselallergie bij Ghanese kinderen onderzocht, en dit is beschreven in hoofdstuk 3. Overgevoeligheidsreacties op voedsel zijn vastgesteld met een vragenlijst en met huidpriktestreactiviteit op pinda's en zes fruitsoorten aanwezig in Ghana. De meest gemelde voedselreacties waren reacties na het eten van bonen, gevolgd door reacties na het eten van ananas en pinda's. In een case-control onderzoek is een deel van de kinderen met positieve reacties op de huidpriktest vergeleken met een controle groep waar geen reacties waren waargenomen. Bij alle kinderen werden IgE antilichamen tegen de voedselallergenen gemeten. Ook werden gerapporteerde overgevoeligheidsreacties tegen voedsel vastgelegd. Er werd een duidelijke associatie gevonden tussen IgE antilichamen tegen specifieke voedselallergenen en de bijbehorende huidpriktestreacties. Deze associatie was sterker bij stadskinderen dan bij plattelandskinderen. Dit onderzoek heeft het belang aangetoond van IgE-gemedieerde overgevoeligheidsreacties op voedsel in Ghanese kinderen en dat er aanzienlijke verschillen bestaan tussen de stad en het platteland met betrekking tot voedselallergieën.

In hoofdstuk 4 ligt de nadruk op pinda-allergie bij Ghanese kinderen. Het interessante is dat pindaconsumptie in Ghana hoog is, terwijl er weinig nadelige gevolgen van pindaconsumptie gerapporteerd zijn. Als maat voor allergische reactiviteit is in dit onderzoek zowel gebruik gemaakt van zelf-gerapporteerde klachten na het eten van pinda's als serum specifiek IgE concentraties en huidreactiviteit als maat voor sensitisatie voor pinda. Onder de studiedeelnemers zijn verhoogde concentraties van IgE-antilichamen tegen pinda-extract gevonden, maar deze verhoging vertaalde zich niet in hogere huidpriktestreactiviteit of zelf- gerapporteerde klachten van pinda-allergie. Bovendien werd er een sterke associatie waargenomen tussen infectie met *Schistosoma haematobium* en IgE-antilichamen tegen pinda-extract. Vanwege deze associatie hebben we van een aantal kinderen de eigenschappen van de pinda-specifieke IgE-antilichamen gekarakteriseerd. We hebben onderzocht of de antilichamen tegen pinda-extract ook zuivere (recombinant) pinda-allergenen (die geassocieerd zijn met pinda-allergie in Westerse landen) konden herkennen. We hebben ook onderzocht of bromelaïne door pinda-specifiek IgE herkend werd. Bromelaïne wordt gebruikt als een marker voor CCD's ('cross-reactive carbohydate determinants'). CCD's zijn suikerstructuren die een gemeenschappelijk onderdeel zijn van allergeenextracten van verschillende bronnen, variërend van planten tot insecten en wormen. Wij vonden dat de IgE concentraties voor zuivere pinda-allergenen heel laag waren, maar IgE concentraties voor bromelaïne juist erg hoog. Bovendien kon de binding van deze IgE tegen pinda-extract door bromelaïne en ook door oplosbaar ei-antigeen van *S. haematobium* onderdrukt worden en vertoonde het weinig biologische activiteit. Het onderzoek in hoofdstuk 4 toont aan dat IgE's tegen CCD's, die mogelijk door eerdere of huidige *S. haematobium* infecties opgewekt zijn, de hoge niveaus van IgE tegen pinda's in Ghanese kinderen zouden kunnen verklaren. Deze IgE-antilichamen tegen pinda hadden een beperkte biologische activiteit, wat overeenkomt met het gebrek aan IgE-gemedieerde pinda-allergie in onze studiepopulatie.

De associatie tussen allergie en immuunreactiviteit op cellulair niveau wordt in hoofdstuk 5 onderzocht. In dit hoofdstuk werd allergie gemeten met huidpriktestreactiviteit op huisstofmijt. Immuunreactiviteit werd gemeten met cytokinereacties in *in vitro* volbloedkweken. Het onderzoek werd gedaan met kinderen die positieve huidpriktestreacties hadden tegen huisstofmijt en met een controle groep die geen reacties vertoonde. In kinderen met een positieve huidpriktestreactie vonden we een versterkte cellulaire immuunrespons.

In de voorgaande hoofdstukken zijn er opvallende verschillen tussen stads- en plattelandskinderen waargenomen met betrekking tot worminfecties en allergieën. In hoofdstuk 6 hebben we daarom onderzocht of er significante verschillen waren in genexpressie-profielen van stads- en plattelandskinderen in onze studiepopulatie. Bloedmonsters van kinderen van een plattelandsschool, en in een stedelijk gebied van een school met een lage sociaaleconomische status (SES) of juist een hoge SES werden gebruikt voor het meten van de expressie van genen gerelateerd aan immuunactivatie en immuunregulatie. We hebben significante verschillen tussen de stad en het platteland gevonden in genexpressie, onder andere voor het gen dat codeert voor de tolerantie-opwekkende signaalstof interleukine (IL)-10. Tegen onze verwachtingen in kon de verhoogde IL-10 genexpressie niet verklaard worden door huidige worminfecties in de plattelandsgebieden. Hieruit hebben we geconcludeerd dat eerdere worminfecties, of andere infecties, mogelijk een rol hebben gespeeld in de verhoogde IL-10 expressie in het plattelandsgebied.

In deze studie zijn ook opvallende verschillen gevonden in genexpressie tussen kinderen van de twee scholen uit het stedelijke gebied. De genexpressie van receptoren die te maken hebben met herkenning van microben en ziekteverwekkers was hoger bij kinderen van de school met hoge SES, vergeleken met kinderen van de school met lage SES. Wij speculeren dat specifieke levensstijlfactoren in onze studiegroep mogelijk een onderdrukkende werking hebben gehad op de expressie van genen die betrokken zijn bij herkenning van microben en ziekteverwekkers. Dit hoofdstuk benadrukt dat expressiepatronen van genen van het immuunsysteem sterk beïnvloed kunnen worden door omgevingsfactoren en dat dit de invloed van verstedelijking op gezondheid zou kunnen verklaren.

Hoofdstuk 7 geeft een overzicht van recente publicaties over worminfecties en allergieën tijdens de kindertijd, gebaseerd op bevindingen van populatiestudies. Dit overzichtsartikel behandelt de volgende onderwerpen: associaties in populatiestudies, het effect van anti-wormbehandeling op allergische reacties, IgE kruisreactiviteit veroorzaakt door wormen en immuun-mechanismen die ten grondslag liggen aan de relatie tussen wormen en allergieën.

De belangrijkste bevindingen van dit proefschrift worden in hoofdstuk 8 samengevat, bediscussieerd en in de context van populatiestudies geplaatst. Het onderzoek naar de relatie tussen worminfecties en allergieën in Ghana heeft inzichten opgeleverd over immuunreactiviteit, IgE kruisreactiviteit en variatie in de symptomen bij allergische

aandoeningen in verschillende geografische regio's in Ghana. Toekomstige studies zijn nodig om voort te bouwen op deze bevindingen, zodat methoden kunnen worden ontwikkeld om allergische aandoeningen te diagnosticeren, behandelen en voorkomen in ontwikkelingslanden zoals Ghana, waar zulke aandoeningen een steeds belangrijker maatschappelijk gezondheidsprobleem worden.

Curriculum Vitae

Abena Serwaa Amoah was born in Roma, Lesotho to Ghanaian parents on 8 February 1977. She completed her secondary school education in Swaziland at Waterford Kamhlaba, United World College of Southern Africa. In 2000, she graduated from Mount Holyoke College in the United States with a bachelor's degree in biological sciences and a minor in anthropology. Following her undergraduate training, Abena worked for two years as a clinical research assistant at Rockefeller University in New York City in the Laboratory of the Biology of Addictive Diseases where she was part of a research team determining neuroendocrine function in individuals with illicit drug addictions. At the end of 2002, Abena relocated to Ghana to gain experience in biomedical research in the tropics. She started with an internship in the Department of Parasitology at Noguchi Memorial Institute for Medical Research (NMIMR) in Accra where she was involved in a new project examining the association between parasitic infections and allergic diseases in Ghanaian children. In 2005, she attained a master's degree in Epidemiology from London School of Hygiene and Tropical Medicine that was funded by the Wellcome Trust. Abena then returned to Ghana and continued to work at NMIMR coordinating field studies for a number of multi-centre studies funded by the European Union that examined parasitic infections and immune responses in Ghana. Her PhD training was a result of ongoing collaborative projects between the Department of Parasitology, Leiden University Medical Center and the Department of Parasitology, NMIMR. Upon completion of her PhD studies, she hopes to continue with research focused on parasitic infections and non-communicable diseases in developing countries.

List of Publications

1. Meurs L, Mbow M, Boon N, Vereecken K, **Amoah AS**, Labuda LA, Dieye TN, Mboup S, Yazdanbakhsh M, Polman K. Cytokine Responses to Schistosoma mansoni and Schistosoma haematobium in Relation to Infection in a Co-endemic Focus in Northern Senegal. *PLOS Neglected Tropical Diseases* (2014) Aug 7;8(8):e3080.
2. Obeng BB, **Amoah AS**, Larbi IA, de Souza D, Uh H, Fernández-Rivas M, van Ree, Rodrigues LC, Boakye DA, Yazdanbakhsh M, Hartgers FC. Schistosoma infection is negatively associated with mite atopy, but not wheeze and asthma in Ghanaian Schoolchildren. *Clinical & Experimental Allergy* (2014) Jul;44(7):965-75.
3. **Amoah AS**, Obeng BB, May L, Larbi IA, Hartgers FC, Boakye DA, Yazdanbakhsh M. Urban-Rural Differences in the Gene Expression Profile of Ghanaian children. *Genes & Immunity* (2014) Jul;15(5):313-9.
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5. Labuda LA, de Jong SE, Meurs L, **Amoah AS**, Mbow M, Ateba-Ngoa U, van der Ham AJ, Knulst AC, Yazdanbakhsh M, Adegnika AA. Differences in Innate Cytokine Responses between European and African Children. *PLOS ONE* (2014) Apr 17;9(4):e95241.
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7. **Amoah AS**, Obeng BB, Larbi IA, Versteeg, SA, Aryeetey, Y, Akkerdaas, J, Zuidmeer L, Lidholm J, Fernández-Rivas, M, Hartgers FC, Boakye DA, van Ree R, Yazdanbakhsh M. Peanut IgE sensitization without skin reactivity or symptoms in Ghana: a role for parasite-induced carbohydrate cross-reactivity. *Journal of Allergy and Clinical Immunology* (2013) Sep;132(3):639-47.
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