ALLERGENS AND ALLERGIC SENSITIZATION IN ASIA AND THE TROPICS

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ALLERGENS AND ALLERGIC SENSITIZATION IN ASIA AND THE TROPICS

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Editorial: Allergens and Allergic Sensitization in Asia and the Tropics

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Keywords: allergen, sensitization, Asia, the tropics, food allergen, house dust mite allergen

Editorial on the Research Topic

Allergens and Allergic Sensitization in Asia and the Tropics

The inception and clinical symptoms of allergic diseases are highly dependent on the environment, which makes the existence of a single pathogenesis for all these processes unlikely. Therefore, the search for common or specific components, whether genetic or environmental, will provide important information about basic mechanisms, phenotype characterization, and personalized management; and for this, nothing better than investigating the pathophysiological and epidemiological contrasts of allergies between populations with different genetic backgrounds or between populations with great environmental differences.

The tropical region has been a traditional source of discoveries from foreign and native scientists, and nowadays interesting projects of scientific groups are exploring several aspects of allergic diseases, among them the patterns of sensitization to common allergens and their impact in the disease manifestations, diagnosis, and treatment. These groups take advantage of known aspects of tropical zones such as their natural characteristics of climate and geographical location, their cultural aspects and the fact that most of the countries are not industrialized.

Besides, Asia is a continent with industrialized temperate and tropical countries, as well as important environmental and cultural particularities that could also influence the development of allergic diseases and, of course, these aspects are currently investigated by several groups from this continent, which are collecting valuable data to resolve recognized unmet needs. In fact, while allergic sensitization and the nature of allergenic sources have been extensively characterized in western industrialized countries, such exhaustive analysis is quite lacking and remains to be broadly performed for Asian and tropical countries.

This "Research Topic *Allergens and Allergic Sensitization in Asia and the Tropics*" includes several representative papers on this underexplored field. Here, different peculiarities of allergic problems in Asia and the Tropics are analyzed, which will surely serve as the basis for making comparisons that help to increase knowledge about their pathogenesis.

Three of these works confirm that patterns of sensitization to pollens, indoor allergens, and food in Asia have regional characteristics that make a difference from western countries. For example, in Korea (Jeong and Park), sawtooth oak, and birch pollens in the spring in conjunction with weed pollens of mugwort, ragweed, and Japanese hop are the main causes of seasonal allergic rhinitis. Among food allergens, the sensitization to silkworm pupa and buckwheat is also common in Korean patients. Interestingly, honeybee venom due to apitherapy is an important cause of anaphylaxis. In a study from Thailand (Katel et al.), an Asian tropical country, indoor and outdoor aeroallergen sensitization, as detected by skin test, was observed in 32 and 7.9% of adult allergic rhinitis patients, respectively. Mono-sensitization was found in 16.9% of patients. Mites (65%) and sedge (39.3%) were the most common indoor and outdoor allergens, respectively. Quality of life was also evaluated, showing that allergic rhinitis has a significant impact on QoL of adult Thai patients.

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In addition, Wai et al. reviewed the sensitization pattern of seafood (fish and shellfish) allergic diseases in Taiwan, Thailand, Singapore, Vietnam, Hong-Kong, and Japan confirming that these Asian countries have unique ways of food processing and dietary habits that explain the observed differences from western countries. For example, fish and shellfish are eaten raw in some countries that may promote sensitization to heat-labile allergens not otherwise seen in other regions. Fermented fish sauce is commonly used as a condiment in some countries which may promote fish sensitization. Shrimp head and shrimp roe are regarded as delicacies in some Asian countries, but their allergen profiles are yet to be characterized. These three studies show how geographical, social and cultural characteristics influence the types of sensitization and their clinical impact, supporting the need of specific diagnostic tools that allow more accurate and personalized management of allergic diseases (1).

In the tropics, there are also particularities that have been analyzed elsewhere (2). Here, two groups from Ecuador and Colombia studied several aspects concerning the emergence of allergic sensitization in children and the emergency room visits of wheezers living in tropical environments respectively. Cooper et al. analyzing a large Ecuadorian birth cohort found that skin prick test positivity starts with mite and is followed by cockroach. The risk of mite sensitization increased with maternal sensitization, while household overcrowding at birth, rural residence, birth order, intestinal helminth decreased this risk. In contrast, Zakzuk et al. had found that indicators of unhygienic conditions were risk factors for house dust mite and Ascaris sensitization in children, using both extracts (3) and recombinant allergens (4). The reason of these contrasting findings deserve a more detailed analysis of the rural vs. urban children populations. Also, Muñoz et al. in this Research Topic present finding indicating that the risk of emergency room visits and intensive care unit admissions for wheezers 2–6 years old increased with poverty indicators such as lack of tap water and sewage, as well as cohabiting with two or more siblings. The origin of these associations was not evaluated but probably depend on respiratory viral infections, which supports the proposal of a more comprehensive and personalized evaluation of wheezing children (5).

Regarding the diagnosis of allergy in the tropics, in their paper, Mourao et al. demonstrate that allergen conjunctival provocation test is safe and reproducible if standardized allergens are available. They use a conjunctival provocation test to define the clinical impact of sensitization to *B. tropicalis* in patients with allergic rhinoconjunctivitis; which is an advance in defining the allergenic activity of allergens and is an important step for obtaining an appropriate diagnostic tools for precision allergology in the tropics (6).

The Research Topic also includes the necessary theme of component resolved diagnosis (CRD) reagents for regional diagnosis of allergy sensitization, and this was investigated by Wangorsch et al. using recombinant *P. americana* allergens and showing that the most frequent inducer of sensitization in a tropical country like Venezuela was Per a 7, a place where sensitization to Per a 2, Per a 5, and Per a 10 was not found. In addition, being mosquito allergy important in the tropics, Cantillo and Puerta discusses the current knowledge about mosquito allergy, allergens, cross-reactivity, and proposals of component resolved approaches based on mixtures of purified recombinant allergens to replace saliva-based or whole-body extracts.

Another important allergen that exhibits cross-reactivity among similar molecules from house dust mite and intestinal helminths such as *Ascaris lumbricoides* and needs to be included in regional platforms for CRD, is glutathione transferase (GST), which is reviewed by Zakzuk et al. GST allergens belong to different classes: mu (Blo t 8, Der p 8, Der f 8, and Tyr p 8), sigma (Bla g 5 and Asc s 13), or delta (Per a 5). In this review, some aspects of the biology of GST, mainly their allergenic activity, structural aspects and the clinical impact of their cross-reactivity are analyzed.

In conclusion, the scientific works of this Research Topic cover different aspects of the pathogenesis, mainly the allergen sensitization, in two regions with geographic, cultural, socioeconomic and genetic particularities; showing interesting results that will help to better understand allergic diseases and improve precision allergology in Asia and the Tropics. Although not the focus of this Research Topic, it is important to keep in mind that the sensitization process is being affected by the climate change (7); therefore we have to be aware of potential changes on the epidemiological trends of allergic diseases.

AUTHOR CONTRIBUTIONS

LC conceived and wrote the manuscript.

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REVIEW
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Allergens of Regional Importance in Korea

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Allergen repertoire should reflect the region's climate, flora, and dining culture to allow for a better diagnosis. In Korea, tree pollens of oak and birch in the spring in conjunction with weed pollens of mugwort, ragweed, and Japanese hop are the main causes of seasonal allergic rhinitis. More specifically, the sawtooth oak in Korea and the Japanese hop in East Asia make a difference from western countries. Among food allergens, the sensitization to silkworm pupa and buckwheat is also common in Korean patients. Honey bee venom due to apitherapy in traditional medicine and Asian needle ant, *Pachycondyla chinensis*, are important causes of anaphylaxis in Korea. Climate change, frequent overseas traveling, and international product exchanges make situations more complicated. Ragweed, for example, was not native to Korea, but invaded the country in the early 1950s. Recently, Japanese hop and Asian needle ants have been recognized as important invasive ecosystem disturbing species in western countries. However, the molecular properties of the component allergens from these unique culprit allergens have been poorly characterized. The present review summarizes the molecular studies on the allergens of regional importance in Korea.

Keywords: native species, invasive species, allergen repertoire, allergen, allergy diagnosis

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INTRODUCTION

Sensitization to an allergen should reflect the exposure from the allergen in the environment. The environment includes climate, which determines fauna and flora, and cultural differences including dining. Different plant and animal species produce different allergen molecules. Some allergens, especially for molecules called pan-allergens, share highly conserved IgE epitopes. However, several differences exist even between allergens that belong to the same protein family. The same species can also produce different isoallergens or isoforms. Different food processing, which reflects cultural aspects, also affects the integrity and modifies the allergenic properties of foodstuffs. In traditional oriental medicine, various local herbs and animal products remain actively utilized for the medicine. Apitherapy (honey bee venom therapy) is a good example of the differences.

These differences in culprit allergens can influence the efficacy of immunotherapy as well as diagnostic sensitivity. However, diagnosis and immunotherapy in Asia rely on the allergen extracts produced from western countries. Some allergens of regional importance are not yet commercially available, and allergy diagnoses depends on cross-reactivity with the extracts from similar species. Furthermore, edible insect allergens should be taken into account since some studies are being performed to develop future diet and animal feed without sufficient investigations on allergic side effects.

This review summarizes the important species in Korea and their cross-reactivity with the representative species. Allergen characteristics will also be discussed if molecular studies have been completed.

INHALANT ALLERGENS OF REGIONAL IMPORTANCE

The 10 most common inhalant allergens (*D. farinae*, *D. pteronyssinus*, *Tyrophagus putrescentiae*, cat epithelium, birch, mugwort, alder, hazel, beech, and oak) account for 90% of inhalant allergen sensitization in a retrospective analysis of skin tests (1). However, many of the positives are not of primary sensitization, but as a result of cross-reactions. A significant increase of skin reactivity over 30 years in Korea was observed with oak (4.7 to 14.4%), birch (7.1 to 13.6%), alder (6.3 to 13.4%), and pine (2.9 to 14.3%) in the 2010s compared to the 1990s (2). Skin reactivity to grass (13.9 to 20.3%) and weed (27.0 to 40.9%) pollens increased, while no differences were observed with house dust mites (55.2 to 55.6%) during the same period.

Tree Pollen

Trees in the spring and weeds in autumn are is the most important causes of pollinosis in Korea. The pollen concentrations of oak in the spring and Japanese hop during the autumn season were the most common in the Korean atmosphere (3). Currently, no pollen extracts from oak and birch native to Korea are commercially available (**Table 1**). Siberian silver birch, instead of common silver birch, is native to Korea. However, birch trees remain uncommon. The most abundant oak species in Korea is the Mongolian oak. Que m 1 from Mongolian oak exhibited better diagnostic value than Bet v 1 (4). However, most Mongolian oaks are found in the mountains with sawtooth oak being common near villages. Moreover, sawtooth oak pollen extracts exhibited stronger allergenic activity compared to Mongolian oak pollen extract (5). Recently, the allergenicity of Que ac 1 from sawtooth oak was investigated (Jeong et al. manuscript in submission). Que ac 1 was shown to be highly polymorphic. The IgE reactivity of recombinant Que ac 1 was more potent than Bet v 1 in Korean oak pollinosis patients. However, no significant differences were displayed in mediator release assays with rat basophilic leukemia cells.

Weed Pollen

Japanese Hop

Autumn pollinosis in Korea is elicited by weed species like ragweed, mugwort, and Japanese hop. Grass causes summer pollinosis, but is rare in Korea. Japanese hop is of particular interest for its high atmospheric concentration in East Asia and recent invasion into Western countries. Interestingly, it exhibited no essential cross-reactivity with a common hop, a closely related species, nor mugwort or ragweed (6–8). However, the molecular details of its allergens have yet to be characterized. A 10 kDa component is known to be a major allergen (7) even though it has not been cloned. Recombinant proteins of profilin, pathogenesis-related 1, polygalacturonase, and pectin methyl esterase, homologous to pollen allergens from Japanese hop, were recognized with 3.4 to 13.8% of IgE antibodies from the patients (9, 10).

Mugwort

Up to 500 different mugwort species have been described worldwide (11). In Korea, 26 mugwort species have been recorded with *Artemisia princeps* being the most prevalent (12). Fortunately, a high sequence identity of three groups of allergens (Art v 1, 2, and 3) from different species and almost equivalent IgE binding capacity of these allergens were reported, allowing diagnosis and immunotherapy with commercialized mugwort extracts (13, 14).

House Dust Mites and Spider Mites

House dust mites are the most frequent cause of allergic diseases (15). Cross-reactivity between house dust mites and storage mites often lead to false-positive reactions to storage mites (16, 17). Polymorphisms of the major allergens from Korean house dust mite isolates (18) were investigated, and sensitization patterns to component allergens were also examined (19, 20). Mite allergic patients suffering from respiratory symptoms were more likely to be sensitized only to Der f 1 and Der f 2 allergens. However, patients suffering from cutaneous symptoms were shown to be sensitized to minor allergens, Der f 11, Der f 13, Der f 14, Der f 32, and Der f Alt a 10 (20). Notably, further studies on IgE reactivity to some of component allergens such as Der p 23, known as a major allergen from Western countries (21), are to be done. Recombinant Der f 23 was recognized by 42.8% of serum IgE from patients, while Der f 2 was recognized by 96.4% (22).

More interestingly, a high sensitization rate to spider mites was described (23). However, the molecular characterization of its allergens has not completed. Cross-reactivity between spider mites and house dust mites should also be performed to verify whether primary sensitization to spider mites is common.

Midges

It is possible that allergic reactions to swarming insect species such as mayflies, stoneflies, and midges can occur. Hemoglobin-like proteins, which help with oxygen uptake in the water, have long been described as the major allergen from the blood worm, chironomid larvae (24). However, these molecules are not found in the adult stage. Tropomyosin, a highly cross-reactive invertebrate pan-allergen, was described as a major allergen from the adult midge of a dominant species (25). A 42 kDa protein was recently identified as a novel allergen from *Cricotopus bicintus*, a hemoglobin-free midge (26).

Companion Animals

Certain dog breeds are being marketed as being hypoallergenic without reliable scientific evidence (27), and some recent studies suggest no evidence of hypoallergenic dog breeds (27, 28). However, the possible different allergenicity of different dog breeds has been described (29–31). Therefore, more investigations are needed on small-sized dog breeds such as the Maltese, Pomeranian, and Poodle, which are popular in Korea. Genetic differences and living environments, including housing situation, diet, and washing habits, may influence the production and accumulation of allergenic substances.

TABLE 1 | Comparison of native and imported commercial plant species for clinical use.

Plant	Imported species for clinical use		Native species	
	Scientific name	**Common name**	**Scientific name**	**Common name**
Birch	*Betula pendula*	Common silver birch	*Betula platyphylla* var. *japonica*	Siberian/Japanese silver birch
Oak	*Quercus alba*	White oak	*Q. mongolica*	Mongolian oak
			Q. acutissima	Sawtooth oak
			Q. dentata	Japanese emperor/Daimyo oak
			Q. aliena	Oriental white oak
			Q. variabilis	Chinese cork oak
Ragweed	*Ambrosia artemisiifolia*	Common/short ragweed	*Ambrosia artemisiifolia*	Common/short ragweed
Mugwort	*Artemisia vulgaris*	Mugwort	*Artemisa princeps*	Korean mugwort
Hop	*Humulus lupulus*	Common hop	*Humulus japonicus*	Japanese hop

FOOD ALLERGENS OF REGIONAL IMPORTANCE

Egg and milk are the most common causes of allergic diseases in Korean children (32), while fruits associated with pollen food allergy syndromes, wheat, and crustaceans are the most frequent causative allergens in Korean adult subjects (33, 34). Interestingly, sensitization to silkworm pupa was most common (25.4%), but the majority of the silkworm pupa-sensitized subjects were asymptomatic to exposure.

Fruits and Food Allergy

Foods (84.8%) are the most common cause of anaphylaxis, followed by drugs (7.2%) in Korean children (35), while drugs (58.3%) are the most common cause, followed by food (28.3%) in adults. The major causative foods of immediate-type food allergy were cow's milk (28.1%), hen's eggs (27.6%), wheat (7.9%), walnut (7.3%), peanut (5.3%), buckwheat (1.9%), and shrimps (1.9%) in children (36). Among the Korean PFAS patients, 8.9% suffered from anaphylaxis (37). The most common cause of anaphylaxis in PFAS was peanut (33.3%), followed by apple (33.3%), walnut (22.2%), pine nut (18.5%), peach (14.8%), and ginseng (14.8%) (38). Foods associated with PFAS are peach (48.5%), apple (46.7%), kiwi (30.4%), peanut (17.4%), plum (16.3%), chestnut (14.8%), pineapple (13.7%), walnut (14.1%), Korean melon (12.6%), tomato (11.9%), melon (11.5%), apricot (10.7%). Interestingly, Korean foods such as taro (8.9%), ginseng (8.2%), sesame leaf (4.4%), bellflower root (4.4%), crown daisy (3.0%), deodeok (3.3%), kudzu root (3.0%) and lotus root (2.6%) also cause PFAS (36). The studies on sensitization pattern to component allergens can provide better picture of cross-reactivity and peculiarity of Korean foods. However, molecular characterizations of major allergens from Korean foods have not been done.

Buckwheat

Buckwheat is a leading cause of anaphylaxis in Korea (39). Various components (9, 16, 19, and 24 kDa) are IgE reactive (40). Among these components, a 16 kDa 2S albumin designated Fag e 2 (41), and a 19 kDa vicilin-like protein designated Fag e 3 (42) are shown to be frequently recognized by IgE from symptomatic allergic patients. However, a 24 kDa protein (Fag e 1), 13 S globulin seed storage protein 3, and legumin are also recognized by asymptomatic sensitized individuals. Recently, a 3.9 kDa antimicrobial peptide designated Fag e 4 and a 55 kDa vicilin-like protein designated Fag e 5 were characterized (43). However, more studies are necessary since only a small number of patients ($n = 7$) were tested for Fag e 4 (5/7) and Fag e 5 (6/7) allergens. Furthermore, Fag e 4 is homologous to hevein and may possibly be cross-reactive with latex allergens.

Fish

Chub mackerels, pollacks, largehead hairtails, redlip croakers, flounders, eels, and anchovies are the most commonly consumed fishes in Korea. Interestingly, a very limited number of fish species, such as codfish and mackerel, are currently utilized to diagnose fish allergies in Korea (**Table 2**). No studies in Korea have been performed into the molecular details of fish allergens. A recent study showed that parvalbumin is the single most potent allergen in Korea, and extensive cross-reactivity among fish species is reported (In press). The high cross-reactivity of parvalbumin allows diagnosis with sibling species. Still, it possible to be sensitized to minor allergens which are not cross-reactive. Therefore, it may be necessary to look into the minor allergens from various fish species in greater detail.

Edible Insects

The edible insect industry is rapidly growing to overcome food shortages associated with population growth (44). However, more attention should be given to possible allergic adverse reactions after the ingestion of edible insects (45).

The Korean government recently created a code for the edible insect industry. In this code, mealworms, silkworms, Rice grasshoppers, rhinoceros beetles, white-spotted flower chafers, and two-spotted crickets are classified as edible insects (**Table 3**). More studies must be performed on mealworm allergens, which are the most frequently consumed insect worldwide. The most commonly identified IgE reactive molecules are tropomyosin, arginine kinase, paramyosin, chitinase, α-amylase, and hexamerin (46, 49–52).

As mentioned above, false-positive reactions (asymptomatic sensitization) to silkworm pupa remain an unsolved problem

TABLE 2 | Comparison of native and imported commercial fish species for clinical use.

Fish	Imported species for clinical use		Native species	
	Scientific name	**Common name**	**Scientific name**	**Common name**
Cod	*Gadus morhua*	Atlantic cod	*Gadus macrocephalus*	Pacific cod
Mackerel	*Scomber scombus* *Scomber japonicus* *Scomber australasicus*	Atlantic mackerel Chub mackerel Japanese/Pacific mackerel	*Scomber japonicus*	Chub mackerel
Cutlass	Not available		*Trichiurus lepturus*	Largehead hairtail
Croaker	Not available		*Larimichthys polyactis*	Redlip croaker
Anchovy	*Engraulis* spp.		*Engraulis japonicus*	Japanese anchovy

TABLE 3 | Edible insects and their potential component allergens.

Insect		Component allergen		
Common name	**Scientific name**	**Protein**	**IUIS nomenclature**	**Reference**
Silkworm (pupa)	*Bombyx mori*	Arginine kinase 27 kDa glycoprotein Tropomyosin Paramyosin Chitinase	Bomb m 1	Liu et al. (46) Jeong et al. (47) Jeong et al. (48) Zhao et al. (49) Zhao et al. (49)
Mealworm (larva)	*Tenebrio molitor*	Arginine kinase Tropomyosin α-amylase Paramyosin Hexamerin/hemocyanin Myosin Trypsin Serine protease		Verhoeckx et al. (50) Verhoeckx et al. (50) Verhoeckx et al. (50) van Broekhoven et al. (51) van Broekhoven et al. (51) van Broekhoven et al. (51) Verhoeckx et al. (50) Verhoeckx et al. (50)
White-spotted flower chafer (larva)	*Protaetia brevitarsis seulensis*			
Two-spotted cricket	*Gryllus bimaculatus*	Arginine kinase Hexamerin/hemocyanin		Srinroch et al. (52) Srinroch et al. (52)
Rice grasshopper	*Oxya chinensis sinuosa*			
Rhinoceros beetle (larva)	*Allomyrina dichotoma*			

for Korean allergic subjects (33). Many studies have focused on cross-reactivity by tropomyosin, mainly from shrimp- and house dust mite-allergic patients, to edible insects. However, silkworm tropomyosin does not have strong allergenicity in Korean patients (48). We identified a 27-kDa glycoprotein as a heat-stable allergen (47) and are working on the characterization of more silkworm pupa allergens.

MISCELLANEOUS ALLERGENS

Stinging Insects

Honey bees, bumble bees, yellow jackets, and hornets are the most frequent causes of insect sting anaphylaxes. They are highly cross-reactive with imported stinging insects even though many of them are different species. The Asian needle ant is of particular interest because it is also regarded as an invasive species to western countries. Pac c 3, antigen 5, is the most important allergenic component as found in other wasps (53). However, it is only partially cross-reactive with Ves v 5, a homologous allergen from yellow jackets (54).

ALLERGEN STANDARDIZATION IN KOREA

In Korea, studies on allergen standardization have been performed with support from the Korean Center for Disease Control and Prevention since 2009 (55, 56). Standardization for the extracts from house dust mites (57), cockroaches (58), and some pollens (mugwort and Japanese hop) (59) was performed and now food allergen standardization is being conducted. Two-site ELISA systems for the quantification of buckwheat allergen Fag e 3 (60) and house dust mite allergen Der f 1 (In press) have been developed. A company with a good manufacturing practice facility for the production of allergen extracts for allergy immunotherapy was established in 2017 (61).

PERSPECTIVES FOR THE DEVELOPMENT OF BETTER ALLERGY DIAGNOSTICS

Challenge test is the most accurate choice of diagnosis, especially for food allergy. However, physicians should take risks of possible adverse reaction including anaphylaxis. Component-resolved

diagnosis (CRD) with recombinant or native allergens could be useful for the identification of primary sensitizers and more accurate diagnosis of allergic diseases (62). CRD can discriminate true food allergy from cross-reactive hypersensitivity in food allergy patients, such as pollen food allergy syndrome (63).

Mediator release from effective cells (mast cell and basophil) is dependent not only on the avidity of IgE antibodies to an allergen, but also on the ability to cross-link FcεR1 and to compete with blocking antibodies (64). The distance between two different IgE epitopes may have an influence on the immune complex shape and aggregation of FcεRI and thus the subsequent activation of basophils (65). Some allergens are not recognized due to its low abundance in the allergenic sources and its extracts. Furthermore, cross-reactive carbohydrate determinant (CCD) often causes false-positive IgE reactions without clinical manifestation (66). Basophil activation test (BAT) could be utilized to overcome these limitations. Some Korean scientists carry out BAT for research purpose, but BAT is rarely utilized in clinical fields. Development of more convenient BAT kit may needed for more common use in clinics.

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Conceptualization and writing of the original draft, and final approval by both authors.

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ORIGINAL RESEARCH
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Conjunctival Provocation Test With *Blomia tropicalis*

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Background: Conjunctival provocation test (CPT) is used to demonstrate clinical relevance to a specific allergen. *Blomia tropicalis* (Bt) is a prevalent allergen in tropical regions. Its major allergen *Blo t 5* is commonly detected in house dust in Brazil. Patients with allergic rhinoconjunctivitis (ARC) have IgE antibodies to Bt although it may not indicate clinical allergy.

Objective: The purpose of this study is to demonstrate the role of CPT in clinical allergy to Bt in allergic conjunctivitis (AC).

Methods: CPT was performed in asymptomatic subjects with ARC ($n = 26$) outside the grass pollen season. They had positive skin prick tests (SPT) to Bt and other common inhalant allergens and they were off topical or systemic antihistamines. Standardized allergens were used for CPT (Blo t 5 462.5 ng/mL in 1:1 solution, Alk Abelló). CPT was conducted on a control group of subjects ($n = 29$) without symptoms of ARC and with negative SPT. CPT was performed with progressive doses of allergen solutions in normal saline (1:32, 1:16, 1:8, 1:4, 1:2). CPT with the same allergen dose that elicited a positive reaction was repeated one week later. The protocol was approved by the local Ethics Board and signed informed consent was obtained from all participants.

Results: There were 92% (24/26) of positive CPT in subjects sensitized to Bt. Significant association was found between SPT and CPT results with Bt ($p < 0.0001$). CPT had 92% sensitivity and 100% specificity when compared to SPT results. Positive reactions with the same dose or one immediately higher occurred in 21 out of 22 subjects who repeated TPC 1 week later. Mild transient nasal symptoms (21/24) were the major side effects of positive CPT followed by moderate periorbital edema which occurred in 41% (10/24). One controlled asthmatic BT-sensitized subject developed wheezing and dyspnea during a positive CPT with Bt that cleared with inhaled albuterol (400 mcg). There were no reactions whatsoever of CPT in non-allergic subjects.

Conclusion: This study demonstrated that Bt may cause allergic conjunctivitis in our population. In addition, CPT is a safe and reproducible test if standardized allergens are used.

Keywords: allergic rhinoconjunctivitis, mite allergy, allergic conjunctivitis, *Blomia tropicalis*, conjunctival provocation test

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INTRODUCTION

Blomia tropicalis (Bt) is a common source of mite allergen sensitization in tropical and subtropical countries causing allergic respiratory diseases such as asthma and allergic rhinoconjunctivitis (ARC). The high temperatures and high humidity levels favor mite growth throughout the year leading to early sensitization and persistent symptoms (1). An increase of ARC prevalence has been observed over the last decades in the Tropics and in Brazil when compared to temperate climate regions, with significant impairment of quality of life, mostly affecting older children (2). Originally classified in the 1970s as a storage mite present in stocked grains, Bt is now recognized as an important indoor allergen. It often coexists with *Dermatophagoides pteronyssinus* (Dp) in house dust samples of patients with ARC in Brazil and other tropical countries (3, 4). Blo t 5 is the major allergen of *Blomia tropicalis* and shares 43% of sequence homology with Der p 5 (5) but low to moderate IgE-cross-reactivity between them is reported (6). Sensitivity to house dust mites detected by skin prick tests or serum specific IgE in ARC is frequent but it may not always reflect clinical allergy (7).

The epidemiology of allergic sensitization was assessed in atopic children and adolescents in Curitiba, Southern Brazil. Skin prick tests to *Blomia tropicalis* were positive in 70.7% of patients with asthma and rhinitis (8).

The ISAAC questionnaire and a previously validated allergic conjunctivitis questionnaire have been applied to 4.520 adolescents. Seven hundred (15.5%) had allergic conjunctivitis and females had a higher prevalence of allergic rhinoconjunctivitis and allergic conjunctivitis when compared to males. There was an opposite allergic sensitization pattern with more IgE sensitized boys than girls. Skin prick tests performed in 472 have shown reactions to Bt in 67% of boys and 48% of girls, respectively (9).

Conjunctival provocation test (CPT) is an investigational tool to assess IgE hypersensitivity on the external ocular surface after the topical application of an allergen in an assumed sensitized subject. It is recognized as the only method to confirm or identify which allergen triggers the signs and symptoms of allergic conjunctivitis. CPT is particularly useful for the etiological diagnosis in persistent allergic conjunctivitis, in multisensitized patients and when sensitization is not concordant with the medical history (10).

The purpose of this study was to demonstrate the role of CPT in the diagnosis of allergic conjunctivitis and that *Blomia tropicalis* is a clinical relevant allergen.

MATERIALS AND METHODS

Patient Population

Twenty-six patients (age range 12–48 years) with symptoms of allergic rhinoconjuntivitis for more than 1 year and Bt-sensitized (positive skin prick tests) were included in the study. They were recruited from the outpatient Allergy Clinic, Hospital de Clínicas, Federal University of Paraná (Brazil). Exclusion criteria included pregnant women and subjects with current active conjunctivitis and/or rhinitis, past or current history of other ophthalmic diseases, active eczema, dermatographism or skin lesions in the areas of the skin tests and patients with unstable asthma. Twenty-nine subjects (age range 13–50 years) without a history of ocular and/or nasal allergic symptoms who tested negative to Bt and other common inhalant allergens by SPT served as the control group. Conjunctival provocation tests (CPT) with Bt were performed in all participants of both groups.

Skin Prick Tests (SPT)

SPT were conducted with standardized extracts of Bt at a concentration of 10 HEP (Alk Abelló—provided by FDA Allergenic, Rio de Janeiro, Brazil) and other common inhalant allergens. Histamine base (5 mg/mL) was used as positive control and diluent (50% glycerin) as negative control. Reactions were graded 15 min later and considered positive if the mean wheal diameter was equal to or >3 mm after the subtraction of wheal diameter of negative control.

Allergen Conjunctival Provocation Test (CPT)

CPT was carried out using progressive doses of allergen solutions as described by Abelson et al. (11). Bt solutions in normal saline at a serial two-fold dilution were prepared daily at room temperature just before each test. Bt allergen extract 10 HEP for CPT had 462.5 ng/mL of Blo t 5 in 1:1 solution as determined by ELISA assay at Indoor Biotechnologies, Charlottesville, USA.

With a pipette, 20 μL of increasing concentrations (1:32, 1:16, 1:8, 1:4, 1:2) of the extract was instilled in the inferior-external quadrant of the bulbar conjunctiva in the right eye every 20 min until a positive reaction occurred and the test was interrupted. The left eye was used as control and received one drop of saline (NaCl 0.9%) initially and then was challenged with a serial two-fold solutions of the diluent (phenol 0.4% and glycerine 50%) in normal saline the same way done with Blomia solutions. A scoring system of severity was used for each ocular symptom. Itching intensity was rated by the patient according to a 0-4-point scale (0 = absent, 1 = intermittent, 2 = permanent awareness but without desire to rub the eye, 3 = permanent awareness but with desire to rub the eye, 4 = the subject insists on rubbing the eye). The other ocular signs were rated by the investigator as following: redness (0 = absent, 1 = localized within some quadrant, 2 = marked or diffuse reddening in the quadrants, 3 = very marked and diffuse reddening in the quadrants), tearing (0 = absent, 1 = slightly wet eye, 2 = some tears, 3 = profuse tearing, tears roll down the face) and chemosis (0 = absent,1 = detectable with slit lamp, conjunctiva raised from sclera, 2 = visually evident raised conjunctiva in limbal area, 3 = ballooning of conjunctiva). The total ocular symptom score (TOSS) was the sum of each individual symptom score. CPT was considered positive when TOSS was ≥ 5, with both redness and itching scores ≥ 2, respectively. TOSS was rated before and 15 min after the instillation of each allergen dose. Patients should be asymptomatic and off any ocular/nasal and systemic antihistamines and corticosteroids for at least 30 days prior to CPT. All provocation tests were conducted outside the grass pollen season.

A second Bt-conjunctival challenge with the concentration that triggered a positive reaction was performed 1 week later to assess CPT's reproducibility.

This protocol was approved by the local Ethics Committee and signed informed consent was obtained from all participants.

Statistical Analysis

The data is presented as numbers and percentages. X^2 test with continuity correction was applied to compare the proportion of SPT and CPT results in both groups. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical and demographic characteristics of Bt-sensitized and control groups are shown in **Table 1**. Females predominated significantly over males in the non-allergic participants.

SPT Results

Of the 26 allergic subjects, two were monosensitized to Bt, 15 had positive SPT to Bt and *Dermatophagoides pteronyssinus* (Dp), eight had positive SPT to Bt, Dp and *Lolium perenne* (Lp) and one reacted to Bt and Lp. SPT were negative to the allergens tested in the 29 controls.

CPT Results

Bt induced ocular and periocular itching within the first minute (median 3.5 ± 1.2 min) of the allergen exposure, reached a peak in 10–15 min and began to fade after 20 min. Conjunctival hyperemia was observed during the first minute (median 6.2 ± 1.6 min) with a peak at 15–20 min. Itching was present in 92% of positive CPT ($p < 0.0001$).

Most of the patients sensitized to Bt (24/26) reacted to CPT. The dose responses to Blo t 5 for positive challenges varied from 28.9 to 231.2 ng/mL (**Figure 1**). One Bt-monosensitized subject had a positive conjunctival reaction to Bt and another did not react. No positive CPT was observed in controls. Positive SPT with Bt was significantly predictive of positive CPT ($p < 0.0001$). Degree of sensitization (mean SPT wheal diameter with Bt allergenic extract) was not correlated with concentration of Blo t 5 to elicit a conjunctival reaction. CPT had 92% sensitivity and 100% specificity for diagnosis when compared to SPT results. CPT induced a positive reaction 1 week later with the same allergen dose in 12/22 subjects and with an immediately higher dose in 9/22. One subject who had an initial negative CPT, did not react to a second challenge.

TABLE 1 | Demographic and clinical characteristics of the study population.

	Allergic	Non-allergic	*P*-values
n	26	29	
Age (years)	12–50	13–50	
Median	25 ± 8.5	34 ± 10.6	$p < 0.42$
Gender (*n*)			
Female/Male	14/12	25/4	$p < 0.0083$
Rhinitis and/or CA	23 (88%)	3 (10%)	
Asthma	1 (4%)	3 (10%)	

CA, allergic conjunctivitis.

Adverse Events

Mild transient nasal symptoms (21/24) were the main secondary outcome of positive CPT followed by moderate periorbital edema in 41% (10/24) of the challenges. One controlled asthmatic Bt-sensitized subject developed wheezing and dyspnea during a positive conjunctival challenge with Bt that cleared with inhaled albuterol (400 mcg). There were no reactions to CPT in non-allergic subjects.

DISCUSSION

This study showed a high rate (92%) of positive CPT in subjects with allergic rhinoconjunctivitis sensitized to Bt and demonstrated that Bt is a causal agent of ocular symptoms. Positive SPT reactions to Bt were highly predictive of positive reactions in the eye ($p < 0.0001$). No positive ocular challenge reactions were observed in the non-sensitized control group. Bt-ocular challenge studies are scarce but similar findings have been described. GarciaRobaína et al. (12) performed a series of conjunctival and bronchial challenges with *Blomia tropicalis* in individuals sensitized to Bt and Dp by SPT or serum specific IgE (s-IgE). There were 62.5% (20/32) of positive CPT with Bt in 18 sensitized subjects and in 2 non-sensitized subjects. Bronchial challenges were positive in 81.8% (9/11) of Bt-sensitized asthmatics. All Dp-sensitized subjects reacted positively to conjunctival and bronchial challenges with Dp except one who was sensitized only to Bt and did not react to the bronchial provocation test. In general, challenges were positive when SPT and/or s-IgE tests were positive but individuals who were sensitized to different mite species might only react to one of them.

Reactions to glycerin and preservatives could account for irritant effect on the ocular surface. In our study, all the procedures were conducted in a control group of asymptomatic

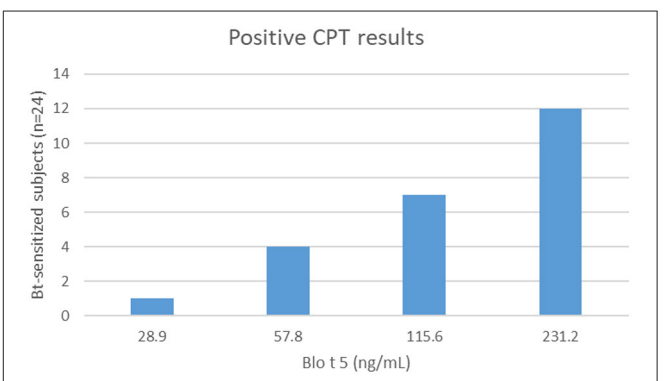

FIGURE 1 | Respondents to conjunctival provocation tests (CPT) and concentrations of Blo t 5.

non-allergic subjects and none of them reacted to the solutions tested. Furthermore, CPT repeated 1 week later induced reactions with the same allergen dose or with an immediately higher dose. The reproducibility of the tests could minimize an irritant effect of preservatives of the extract.

Stanaland et al. (13) demonstrated responses to nasal challenge to Bt in 83% of Bt-sensitized subjects. In their region, Bt was found in 33% of house dust samples in concentrations >150 mites per gram of dust (14) and sensitization to Bt detected by SPT accounted for 38% of allergic respiratory symptoms (15). No positive nasal challenge reaction to Bt was found in the group of individuals sensitized to other species of house dust mites such as *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. Nevertheless, Bt was allergenic and should be considered as a cause of allergic rhinitis.

In Brazil, Barreto et al. (16) demonstrated the allergenicity of Bt by nasal challenges (NPT) in children with perennial allergic rhinitis who were sensitized to Bt and Dp. Specific and non-specific nasal mucosa reactivity were assessed. There were 60% of positive challenges to Bt and 90% to Dp. Eight out of 10 histamine NPT were positive showing a high prevalence of non-specific hyperreactivity of the nasal mucosa in children with allergic rhinitis. Conjunctival hyperreactivity to non-specific stimuli has also been documented in allergic and non-allergic patients (17). CPT with hyperosmolar solutions in patients with ocular symptoms have elicited conjunctival hyperemia, mild itching/burning and tearing in 84% of allergic patients sensitized to dust mites and grass but 16% of non-allergic subjects also had positive ocular challenges to glucose solutions (18). Allergic subjects exhibit more conjunctival responsiveness than non-allergic subjects, even when asymptomatic, probably due to a minimal persistent inflammation process. We could speculate that the perennial exposure to house dust mites might be a factor that could contribute to conjunctival inflammation and hyperreactivity.

The frequency of sensitization to *Blomia tropicalis, Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in asthmatics from different cities in Latin America have been reported between 60 and 97%. *Blomia tropicalis* is considered a common sensitizer in Brazilian atopic children (19). In patients with atopic dermatitis, having sensitization to rBlo t 5 is highly specific and sensitive although there was also high sensitization to the components nDer p 1/n Der f 1 in severe forms of atopic dermatitis (20).

In general, CPT with Bt was a safe procedure triggering self-limited ocular and nasal symptoms mainly related to the early phase reaction of Ige-allergic inflammation. Only one asymptomatic asthmatic patient developed mild wheezing during CPT. A study realized in Singapore with nasal challenges with Bt provoked late-phase reaction wheezing in patients with allergic rhinitis and a history of asthma (21). Even though most adverse reactions of CPT are mild, there is potential for more severe/systemic responses and it should preferably be performed in centers where side effects can be handled (22).

Hypersensitivity to *Blomia tropicalis* is usually based on the results of SPT or serum levels of specific IgE to the whole allergen or to Blo t 5, its major allergen (23–25). Despite the frequent sensitization observed in SPT and S-IgE, Blo t 5 concentration in house dust samples in Brazil (26) and in other tropical climate countries (27) have been found to be low with predominance of Dp or other mite species. Another source of discrepancy in this issue could be the existence of different regional variants or isoforms of Blo t 5 that could be underdetected by the ELISA monoclonal assays. Relative abundance, instability of Blo t 5 or reliability of the assay used may account for these findings.

One limitation of this study was that standardization was solely based on Blo t 5 allergen content, other Bt allergens have been reported to contribute to the allergenic activity of Bt extracts and usually Blo t 5 is only a minor fraction of the protein content in these products (3, 23).

The small number of subjects and the predominance of females in the control group could both be confounding factors. Target organ challenge studies may demonstrate a specific allergen as the sensitizer and the trigger of symptoms (28). In our study, Bt-extract was standardized and the concentration of Blo t 5 that elicited signs and symptoms of allergic conjunctivitis was known. The results are strengthened by the biological effect of the extract and the dose-response behavior, although it could be misleading to attribute all or most effect solely to Blo t 5. It is essential to use standardized allergens for conjunctival challenges to obtain accurate and reproducible responses of true sensitization (29). From a clinical perspective, CPT could be useful to select allergen extracts for SPT and immunotherapy.

CONCLUSION

This study demonstrated that Bt may cause allergic conjunctivitis in our population. In addition, CPT is a safe and reproducible test if standardized allergens are used. SPT is an indicator of clinical relevance of sensitization in patients with allergic conjunctivitis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comite de Etica em Pesquisa em Seres Humanos Hospital de Clinicas–UFPR. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Allergological Importance of Invertebrate Glutathione Transferases in Tropical Environments

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Glutathione-S transferases (GSTs) are part of a ubiquitous family of dimeric proteins that participate in detoxification reactions. It has been demonstrated that various GSTs induce allergic reactions in humans: those originating from house dust mites (HDM), cockroaches, and helminths being the best characterized. Evaluation of their allergenic activity suggests that they have a clinical impact. GST allergens belong to different classes: mu (Blo t 8, Der p 8, Der f 8, and Tyr p 8), sigma (Bla g 5 and Asc s 13), or delta (Per a 5). Also, IgE-binding molecules belonging to the pi-class have been discovered in helminths, but they are not officially recognized as allergens. In this review, we describe some aspects of the biology of GST, analyze their allergenic activity, and explore the structural aspects and clinical impact of their cross-reactivity.

Keywords: allergen, house dust mite, Ascaris, cockroach allergen, glutathione S-transferase, IgE, tropics

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INTRODUCTION

Glutathione-S transferases (GSTs) are a part of a ubiquitous family of dimeric proteins that have triggered the interest of different biology-related disciplines due to their involvement in drug resistance, carcinogenesis, and allergy. Most of these isoenzymes participate in detoxification reactions, coupling reduced glutathione (GSH) to xenobiotics and/or endogenous substances, such as bilirubin and steroids. They have allergological importance for multiple reasons: several of them are epidemiologically relevant as environmental sensitizers and due to their sequence and structural conservation even among phylogenetically distant species, they may mediate cross-reactivity among invertebrate allergen sources, such as cockroach, house dust mites (HDM), and helminths. Also, probably because of their abundance and immunogenicity, some helminth GSTs have been evaluated in pre-clinical studies and clinical trials as vaccines to prevent helminthiasis (1, 2). Altogether, it means that it is necessary to determine the allergenic activity of these IgE-binding molecules and the clinical impact of their cross-reactivity, including potential allergic reactions after anti-helminth vaccination. Although it is still an unexplored field, immunomodulatory properties have been detected in certain parasite GSTs, of which some of them interfere with type 2 responses or strengthen immunosuppression (3–5). In this review, we describe some aspects of the biology of GST, analyze their allergenic activity, and explore the structural reasons and clinical impact of their cross-reactivity.

BIOLOGICAL ASPECTS OF GSTs

The main enzymatic reaction of GST consists of the conjugation of the thiol group of GSH to chemical electrophilic centers. The resulting conjugates are less reactive or more water-soluble, facilitating its excretion (6–8). Other less common functions of GST have been described in invertebrates. Meyer et al. purified a sigma-GST from the helminth *Ascaridia galli* with homology to a GSH-dependent prostaglandin-H D-isomerase and high activity and specificity in the GSH-dependent isomerization of prostaglandin H to prostaglandin E, a lipid mediator associated with suppression of host immunity (9). Two kappa-GSTs from *Caenorhabditis elegans*, were located in peroxisomes and mitochondria, and, by RNA interference experiments, were found to be involved in oxygen consumption and lipid metabolism (10). Some GSTs also catalyze the selenium-independent reduction of peroxide-containing compounds (11). Arthropod GSTs may confer resistance *via* direct metabolism, sequestration of chemicals, or metabolizing secondary products. To date, GST activity has been associated with resistance to all main classes of insecticides (12). In helminths, the main detoxifying system is mediated by GSTs; CYP450, another important system found in animals, is not present in most parasites (13). GST content and activity are usually investigated through affinity chromatography and using 1-chloro-2,4-dinitrobenzene (CDNB) substrate, since most of these enzymes may catalyze reactions. However, due to their importance in insecticide and worm chemotherapy resistance, it is common to use high-throughput methods, such as quantitative transcriptomics, functional genomics, and structural studies, for their characterization (12).

Classification

Members of this family are classified according to their cellular localizations into three major families, i.e., cytosolic, mitochondrial/peroxisomal, and microsomal GSTs (14). Cytosolic GSTs are the most abundant and best characterized. In general, they are 23–28 kDa molecules that are functionally active as dimers. They contain the fundamental signature of an N-terminal domain that binds GSH and an alpha-helix conformation in the C-terminal domain, also known as the substrate-binding site. Among cytosolic GSTs, several classes (mu, alpha, pi, theta, sigma, zeta, and omega) are widely distributed in nature. Others are more specific to certain kingdoms or phyla. Epsilon GSTs, for example, have been identified only in insects and delta GSTs in certain arthropod classes (15). All the known allergens belong to mu, sigma, or delta classes. Also, IgE-binding molecules belonging to the pi-class have been discovered in helminths (16), but have not been officially recognized as allergens by the IUIS. Although most GSTs conserved a general structural pattern, isoforms from different classes present low sequence similarity (14, 17). It is expected that GSTs from different classes are not cross-reactive (18), but this is not a rule; for example, cross-reactivity between the sigma Bla g 5 and a pi-GST from the nematode *Wuchereria bancrofti* was demonstrated (16). Knowledge about the class properties of GSTs may guide the research on its allergenic aspects: (a) confirmation of enzymatic activity of a recombinant GST supports a well-folded product, similar to the natural molecule; (b) it must be considered in the evaluation of intrinsic features of allergens that may promote Th2 responses far more than its IgE-binding properties; (c) the natural abundance of GST isoenzymes according to the species is helpful to evaluate the potential cross-reactivity with other sources and understand its clinical impact. For example, a delta-GST from a cockroach may cross-react with an ortholog in HDM (19), but in this last organism, the isoform may not be abundant (20).

Sigma Class

Members of this class have been described in mammalians, chickens, insects, helminths, and mollusks (21). The cockroach allergen Bla g 5 (22) and the nematode GST Asc l 3 (23) belong to this class. Their X-ray crystal structures confirm their similarity with other sigma GSTs (24). The first mammalian sigma-class determined structure was from the rat hematopoietic prostaglandin D synthase with GSH bound (25). Human sigma GST is involved in the biosynthesis of prostaglandin D2 (PGD2) from prostaglandin H2 (PGH2) (21). Also, the *Schistosoma hematobium* antigen has been found to retain this enzymatic activity, and murine models have supported the concept that PGD2 production inhibits host immune response and favors parasite survival (26).

Mu Class

Its members have a particular loop between the β2 sheet and the α2 helix, and they typically attach GSH to highly electrophilic compounds (27). All described mite GSTs (Der p 8, Der f 8, Blo t 8, and Tyr p 8) correspond to the mu-class (24). Even though GSTs from this class have been reported in different helminth species, there is no description of a mu-GST allergen in helminths (28, 29). In *Fasciola hepatica*, the common liver fluke, mu-GST expression is highly reactive to chemotherapy, and its activity is involved in protection against xenobiotics, even in a more important way than its sigma member (29). Vaccination with a DNA construct coding for this GST induced humoral response of different isotypes, including IgE but was dominated by the IgG1 isotype (30).

Delta Class

Together with epsilon, these GST classes are monophilic for arthropods. Delta is the major cytosolic GST class in insects and their genes are organized in large clusters (31). Allergenic delta-GSTs have been identified in cockroaches (32, 33). Their presence in other arthropods different from insects has not been fully investigated, although there are reports of delta-GSTs in Arachnida, including the Acari subclass (34, 35). Dougall et al. identified a delta-like GST in *Dermatophagoides pteronyssinus*, but this sequence also showed similarity with epsilon-GSTs (20). Classification of GST may be imprecise since certain proteins may have homology to more than one class of GST. In this case, studies of the gene locus, substrate-specificity experiments, and evidence of cross-reactivity with a specific class may help to better define its classification. Insects tend to have numerous delta-GST paralogous genes (34). For example, delta class represents

39% of all GST genes in *A. gambiae* (31). On the contrary, in Acari, mu-GSTs tend to be relatively more abundant (35). Delta-GST activity has been linked to resistance to insecticides and acaricides. Their expression may also be raised by exposure to toxic compounds (36). This has also raised the concern of higher allergenic activity (37) of HDM in cities with poor air quality associated with diesel and other environmental contaminants, as described for Der p 8 (38).

ALLERGENS

It has been demonstrated that several GSTs induce allergic reactions in humans, those originating from HDM, cockroaches, and helminths being the best characterized (**Figure 1**). After a systematic search in MEDLINE using the words "glutathione transferase," "allergen," and "IgE," we identified 24 out of 229 references reporting information about IgE-binding properties in 12 GSTs. When searching in Allergome.org, we identified 30 entries with this biochemical function belonging to different sources: cockroaches (32, 33), HDM (37, 42, 43), scabies (20), fungi (44), plants (45, 46), and helminths (23). Only 10 of them are officially recognized as allergens by the WHO/IUIS Allergen Nomenclature Committee and eight are derived from invertebrates. Two GST from helminths (Asc s 13 and Asc l 13) are officially recognized allergens, but in total, eight molecules are listed, of which three are predicted *in silico* as allergenic without any experimental evidence of at least IgE binding. **Table 1** shows the most important features of the eight invertebrate GSTs officially reported as allergens, showing that most of them have positive provocation assays, confirming their allergenic activity. Mueller et al. solved the 3D structure of four of these allergenic GSTs and confirmed the global structural similarity among them; however, a relatively low level of conservation of surface-exposed residues was observed (24). Except for Der p 8 and Der f 8, the allergenic activity (47) of most of them have been evaluated by *in vivo* or *in vitro* provocation tests (**Table 1**), suggesting that they may have a clinical impact. However, their involvement in disease presentation has not been assessed (i.e., case-control studies or avoidance studies). It would be interesting to explore other non–IgE-mediated effects of GST that may predispose to allergic inflammation. Evidence of this was obtained for Der f 8 as explained below (48). Also, immunomodulatory properties influencing type 2 responses have been described for a helminth GST from *Schistosoma* that reduces intestinal inflammation through eosinophil-dependent modulation of pathological Th1 responses (49).

House Dust Mites

Dermatophagoides pteronyssinus

Described by O'Neill et al. (50), Der p 8 was the first reported GST allergen (Der p 8.0101). In this review, an IgE-binding frequency of 40% in 193 *D. pteronyssinus* allergic patients was observed (43). Another isoform was isolated and produced by research group of Dr. YK Chua; interestingly, due to only six amino acid replacements, this isoform had an isoelectric point (pI) of 8.5, which is significantly different from the 6.1 predicted pI of Der p 8.0101. Characterization of the native GST fraction by 2D-gel electrophoresis and western blot analysis (with anti-Der p 8 polyclonal antibodies) indicated that there were at least eight GST isoforms and confirmed the existence of the cloned allergen. Taiwanese patients showed 96 and 84% of IgE reactivity with native and recombinant Der p 8, respectively. Results from Malaysia and Singapore showed lower sensitization rates: 65 and 75%, respectively. Huang et al. also reported moderate cross-reactivity between Der p 8 and the American cockroach native GST isolated by affinity chromatography (42). We also performed data mining of the *D. pteronyssinus* published genome, finding eight entries sharing different degrees of sequence identity with Der p 8.0101 (41–99%) and were annotated as mu-like GSTs. Characterization of their biochemical features by ProtParam indicated that their *pI*s also ranged from five to nine as the results previously observed at the proteomic level (42). It is expected that, as in most arthropods, HDM harbor genes coding for other GST classes. Dougall et al. (20) by a random screening of clones from a cDNA library, found a delta-like GST sequence (Dp7018E11) with only 26% identity to Der p 8.0101. Furthermore, Liu et al. identified an IgE-binding spot compatible with this sequence after 2D separation of the whole *D. pteronyssinus* extract that was also identified in the genome sequence of this mite (51). There are no reports about the evaluation of its allergenic activity, in neither *in vivo* nor *in vitro* tests.

D. farina

Der f 8 was reported by An et al. (52). Serology studies indicate IgE-binding frequencies to Der f 8 in the range of 6–41% (53, 54), but there is no information about its allergenic activity, neither *in vivo* nor *in vitro* tests. An interesting data about Der f 8 is its capacity to induce T cell immunoglobulin mucin domain 4 (TIM4) RNA expression in bone marrow-derived dendritic cells at even higher levels than Der f 1 or Der f 2 (48). The interaction of TIM4 with its cognate ligands, TIM1, can promote Th2-cell proliferation, and the blockade of these molecules remarkably dampened Th2 differentiation and allergic reactions (55, 56). Mice sensitized and challenged with Der f 8 developed a strong pulmonary inflammation and increased local and systemic production of Th2 cytokines. Depletion of GST from HDM extracts abrogated the inflammatory potential normally observed with the complete extract and induced regulatory T cells (48).

Blomia tropicalis

Blo t 8 is a 23 kDa allergen with two different sequence entries (sharing 99% identity) in UniProt, and two reported IgE-binding frequencies of 10 and 80% in Singapore and Colombia, respectively (57, 58). The tertiary structure of Blo t 8.0101 was experimentally defined by Mueller et al. and matches with a typically mu-GST (24). Blo t 8 can induce positive skin test reactions (23) and also induce positive passive cutaneous anaphylaxis tests (59). Acevedo et al. described in the natural extract of *Ascaris suum*, a 23 kDa band inhibited by the mite extract. Further MALDI-TOF experiments identified this band as a GST. Blo t 8 and Der p 8.0101 have 35.5% identity. The cross-reactivity between Blo t 8, Der p 8, and Asc l 13 has not been experimentally demonstrated. *In silico* analysis

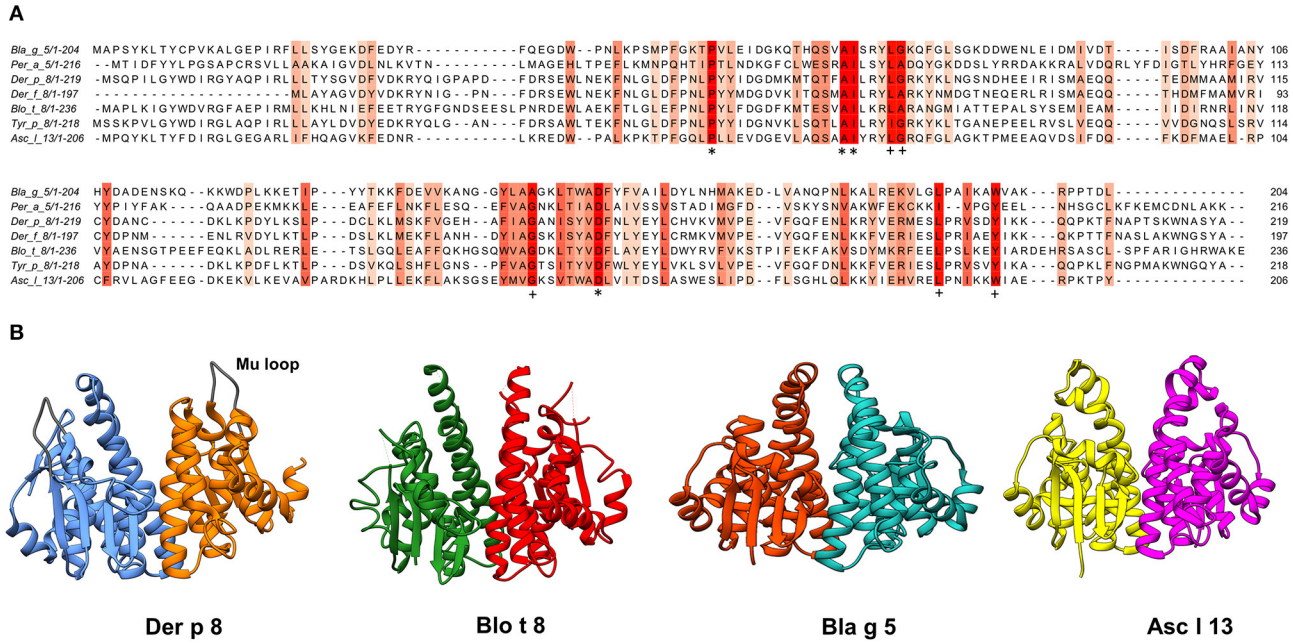

FIGURE 1 | (A) Multiple sequence alignments of reported GST Allergens. The most conserved columns have the most intense color, and the least conserved are the palest. A threshold score of 6 was used (conserved amino acids are those with six or more shared properties out of 10) (39). Columns with the same amino acids are marked with "*", and columns with mutations where all properties are conserved are marked with "+". AMAS method of multiple sequence alignment analysis was used to calculate the physicochemical properties conserved for each column **(B)** Ribbon diagrams of the structures of allergenic GSTs were generated in UCSF Chimera (40) using data from the PDB database (41), Der p 8 (PDB: 4Q5Q), Blo t 8 (PDB: 4Q5N), Bla g 5 (PDB: 4Q5R) and Asc l 13 (PDB: 4Q5F).

TABLE 1 | The allergenic activity of glutathione-S transferase (GST) from invertebrates officially named as allergens.

Allergen	Species	Class	Provocation test *in vivo*	Provocation test *in vitro*	PCA animal model	Non-IgE–induced inflammation
Bla g 5	*Blattella germanica*	Sigma	ST	n.d.	Yes	n.d.
Per a 5.0101	*Periplaneta americana*	Delta	n.d.	BAT	n.d.	n.d.
Per a 5.0102		Delta	n.d.	BAT	n.d.	n.d.
Der p 8	*Dermatophagoides pteronyssinus*	Mu	n.d.	n.d.	n.d.	n.d.
Der f 8	*Dermatophagoides farinae*	Mu	n.d.	n.d.	n.d.	Yes
Tyr p 8	*Tyrophagus putrescentiae*	Mu	n.d.	HR	n.d.	n.d.
Blo t 8	*Blomia tropicalis*	Mu	ST	n.d.	n.d.	n.d.
Asc s 13	*Ascaris suum*	Sigma	ST	n.d.	n.d.	n.d.

ST, skin prick test; HR, histamine release; BAT, basophil activation test.

of the 3D structures of these molecules suggest that cross-reactivity between them is low (24). In summary, the allergenic activity of Blo t 8 is confirmed, but more information about its importance on allergic sensitization in tropical populations is needed due to the high variations in seroprevalence between Asia and Latin America. There is evidence that helminth infections boost IgE response to cross-reactive environmental allergens, such as HDM and cockroaches (16, 60) and, possibly, the national prevalence of helminthiasis plays a role in this difference on Blo t 8 sensitization rates. In Singapore, a high-income country, ascariasis is infrequent, in contrast to Colombia where this infection occurs in >20% of the population (61, 62).

Storage Mites

Tyr p 8 was isolated from *Tyrophagus putrescentiae* by affinity chromatography. It is a 26 kDa protein sharing 83% of amino acid sequence identity with Der p 8. IgE seroprevalence against Tyr p 8 was 45.3% (48/106) among Taiwanese allergic patients who were sensitized to the mite extract. Pre-adsorption of all sera with *D. pteronyssinus* extract reduced the rate of IgE recognition to 18%, which suggests cross-reactivity among GSTs from these sources. There is evidence of basophil histamine release sensitized with sera from Tyr p 8-allergic patients, confirming its allergenic activity (63).

Cockroach

Blatella germanica

Bla g 5, the GST from *B. germanica*, is one of the most common causes of sensitization among cockroach-allergic patients. Arruda et al. first isolated Bla g 5 by affinity chromatography from the natural extract. Serology analysis revealed 67.5% of sensitization among 40 asthmatic patients reactive to this cockroach (22). This group also cloned the sigma GST (Bla g 5.0101), which is experimentally confirmed as a GST and is officially recognized as an allergen. Interestingly, Bla g 5 is the only allergen with a high correlation between specific IgE and wheal size in the skin test (64). Satinover et al. measured using the CAP assay IgE reactivity to different cockroach components, demonstrating in 118 patients that, together with Bla g 2, Bla g 5 dominates IgE responses (65). In summary, this allergen is an epidemiologically relevant component, whose allergenic activity has been confirmed *in vivo* by skin tests.

Jeong et al. isolated a delta-Bla g 5 isoform with just 15% identity with the sigma class and limited cross-reactivity. This isoform showed higher enzymatic activity when CDNB and 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane and 4-hydroxynonenal were used as substrates, but its IgE-binding frequency was lower (17.9%) compared to the sigma class (20.5%) in cockroach-sensitized patients from Seoul, Korea. There is no evidence of its allergenic activity (33).

It would be interesting to explore whether there is a relationship between low socioeconomic background and higher sensitization to Bla g 5, as it has been observed for the complete German cockroach extract (66). Possibly, due to lower hygienic conditions, higher environmental levels of *B. germanica* allergens are found in low-income dwellings, as detected with Bla g 2 in the United States (67). Since helminth infections are linked to poverty, it may be hypothesized that underdeveloped countries may have higher sensitization to allergens with cross-reactive homologs present in helminths. Santiago et al. demonstrated that filarial-infected patients had higher IgE levels to Bla g 5 than non-infected donors living in the same endemic regions (16); however, this is not easy to explore because of variations in its geographical distribution. *B. germanica* is one of the most common cockroach species found in Canada, Europe, and the United States (68). Despite being a cosmopolitan species and its origin being tropical Asia (69), it is present but not abundant in the tropics. Meanwhile, in tropical and sub-tropical regions, *Periplaneta americana* is the most prevalent indoor species (70). Barbosa et al. found only 7% of sensitization to sigma Bla g 5.0101 in 53 subjects in Brazil (71).

Periplaneta americana

A GST-like sequence from this cockroach was reported in 2006 in GenBank by Chew et al., but without data about its allergenicity. PCR primers based on this sequence led to the identification of the two isoforms with 99% homology by different groups, officially named as Per a 5.0101 and Per a 5.0102. Wei et al. produced the 0.0102 isoform as a recombinant protein in Baculovirus and *E. coli* systems. Chinese *P. americana*-allergic patients showed 25% (4/16) of IgE reactivity to this isoform. Both recombinant versions induced basophil activation as measured by the CD63-based flow cytometry assay (19). Per a 5 was described as a delta-GST with 81% homology to BGGSTD1 and low similarity to sigma GST allergens (15 and 13% to Bla g 5 and Der p 8, respectively). Sookrung et al. isolated Per a 5.0101 from *P. americana* collected in a dwelling in Thailand and characterized its whole repertoire of GST isoenzymes. They found that native and recombinant *P. americana*-GSTs were enzymatically active and 100% of IgE reactivity in sera of all *P. americana*-allergic patients living in Thailand (32). Proteomic analysis indicated that the native GST comprises three isoforms of delta and sigma classes. Interestingly, all isoforms interacted with serum IgE of the cockroach-allergic subjects. IgE reactivity to the original sequence published by Chew et al., (98–99% similar to the other two official isoforms) was 47% in Indian allergic patients. In this same review, it is reported that Per a 5 inhibited IgE binding to cockroach and HDM extracts (72). Per a 5 was also tested in Taiwan with no differences in IgE reactivity between allergic rhinitis (66%) and asthmatic (55%) patients (73). In summary, *P. americana* GST is an allergen with two isoforms with confirmed allergenic activity and epidemiological importance in tropical Asia. Data from Latin America is missing.

Helminths

Different helminth GSTs (*S. haematobium, S. mansoni,* and *W. bancrofti*) can induce IgE antibodies in infected individuals (16, 74, 75), but only *A. lumbricoides* and *A. suum* GSTs have been reported as allergens. In the tropics, *A. lumbricoides* is of great relevance; first, it causes the most common soil-transmitted helminthiasis (61); and second, several epidemiological surveys have found that ascariasis is a risk factor for asthma and atopy (76). We cannot rule out the importance of other helminths GSTs as allergens since this has not been systematically explored.

Ascaris spp.

The native *Ascaris lumbricoides* GSTs (nGSTA) purified by Acevedo et al. contained six isoforms that bind IgE (23). To date, the best-studied of these isoforms is the sigma class GST, currently reported as the Asc l 13 allergen by the WHO/IUIS Allergen Nomenclature Sub-Committee. Its sequence is completely identical to Asc s 13. The allergenic activity of the recombinant isoform (rAsc s 13) and nGSTA have been evaluated in asthmatic patients exposed to *A. lumbricoides*. In this population, there was a sensitization frequency of 19.5% in asthmatic patients and 13.2% in controls to rAsc l 13; and the strength of IgE levels to rAsc l 13 among asthmatic patients was significantly higher compared to mite and cockroach GSTs. Additionally, four out of 10 asthmatics had a positive skin test to nGSTA, proving the allergenic activity of this molecule and its possible clinical relevance for some patients (23).

Results from the birth cohort study FRAAT (Risk Factors for Asthma and Atopy in the Tropics) conducted in a deprived community living in Cartagena, Colombia, indicated that ∼20% of children were sensitized to rAsc l 13 at a young age (6 months old), increasing to 45% at 3 years of age. A positive IgE response to Ascaris GST was associated with housing features related to poor hygienic conditions and Ascaris infection (77). Also, a de-sensitization pattern to rAsc l 13 was evident with a 25%

lower rate at 6 years of age. This process of desensitization may continue to older age, according to the results of Acevedo et al. in asthmatic patients living in the same city. We found that the frequency of IgE response to rAsc l 13 was significantly greater in children (7–13 years) compared to adolescents and adults (14 years and older) (23). In that sense, IgE sensitization to rAsc l 13 may be associated with exposure to Ascaris, which tends to be more frequent during infancy (77). It is still necessary to further characterize the allergenic activity of Ascaris GSTs and their clinical relevance.

To characterize other allergenic isoforms of Ascaris ssp. in our laboratory, we identified the GST genes predicted by the Davis laboratory at the University of Colorado and expressed them as recombinant proteins. We have analyzed the enzymatic activity and humoral response of an omega-class isoform. The recombinant GST omega-class (rGSTO) has dehydroascorbate reductase and thiol transferase activity reported as well for other omega-class GSTs (51). More than 30% of people from a rural area and endemic for *A. lumbricoides* had IgE against GSTO with a similar frequency to rAsc l 13 (78). Despite corroborating that this sequence codes for a biologically active product, in the correction of the *A. suum* genome assembly submitted by the authors in 2018, the current sequence coding for one omega-GST (F1LF49) has only 90.3% of identity to rGSTO.

In 2018, the Parasite Genomic group at the Wellcome Trust Sanger Institute made the draft genome assembly and gene predictions of *A. lumbricoides* using a strain from the Republic of Ecuador. Based on this work, we identified 15 GST sequences of *A. lumbricoides* reported in the UniProt database. The sequence and tertiary structure were verified to confirm that these proteins belonged to this protein superfamily. Most predicted products from these sequences had features of the cytosolic subfamily, as the thioredoxin-like fold domain and the GST N-terminal and C-terminal domains. There was only one sequence identified as kappa subfamily GSTs, due to the presence of the DSBA-like thioredoxin domain, and one as microsomal subfamily GST (79). In *A. suum*, according to the genome published by the Davis Laboratory at the University of Colorado in 2018 (80), we identified 14 GSTs in the UniProt database. Most of the proteins have features related to the cytosolic subfamily, two have features of the kappa subfamily and one has features of the microsomal subfamily. Cytosolic GSTs from *A. lumbricoides* and *A. suum* were classified by their homology with different GST classes (**Figure 2**). At the transcriptional level, we found expression data for 10 out of the 11 cytosolic GSTs (**Figure 3**). It is generally observed that one pi- and one sigma-isoform (which we also confirmed in the cDNA library) are the most abundant (FILV84 and P46436, respectively). The transcriptional expression also differed between tissues and developmental stages of the parasite. *A. suum* and *A. lumbricoides* are identical at the morphological level, but not in their genomes. DNA sequences coding for GST isoforms in *A. lumbricoides* were in the range of 77.5 and 99.5% identity with another GST from *A. suum*. Only one GST (A0A0M3I3X4) of *A. lumbricoides* did not have a similar sequence in *A. suum* and two sequences in *A. suum* (F1LBW9 and F1LCY4) were not identified in *A. lumbricoides*. The *A. suum* kappa-GST (F1LDD6) had an identity of 68.3% with an *A. lumbricoides* DSBA domain-containing protein with a match length of 182 of 254 amino acids.

IMMUNOMODULATION

Several investigations have pointed out different parasite GSTs as immunomodulatory proteins, including molecules derived from helminths and protists (83). The participation of GST on immunoregulation in mammalian species has also been documented (21). As the focus of this review is invertebrate GSTs, we describe only the evidence on helminth GSTs showing any effect on the immune responses of exposed hosts.

Immunomodulatory properties of sigma-GST (fhGST-si) from *F. hepatica* have been found over mouse BMDCs, reducing their inflammatory response to LPS and inhibiting its capacity to induce Th17 polarization (3). Stimulation of BMDCs with the fhGST-si induced secretion of pro-inflammatory cytokines, such as IL-6, IL-12p40, and MIP2 e IL-10, but caused a partial cellular activation: CD40 upregulation in the absence of CD80, CD86, and MHC-II overexpression. Priming dendritic cells with fhGST-si before the addition of OVA reduced Th17 responses in DO11.10 mice, a transgene strain reactive to an OVA peptide antigen. This same group also tested the recombinant mu-GST from *F. hepatica*, with no observable immunomodulatory effect of this isoform. Aguayo et al. tested the native *F. hepatica* GST (nFhGST), which is majorly comprised of mu-class GST isoforms (96%), in a septic shock mouse model. It was observed that nFhGST significantly suppressed the LPS-induced TNFα and IL1β *in vitro* production by macrophages and the pro-inflammatory cytokine/chemokine storm within C57BL/6 mice elicited by lethal doses of LPS (84).

P28GST, a GST derived from *Schistosoma haematobium* has shown to induce a strong mucosal immune response, associated with IL-10 and Th2 cytokine production in animal models and humans (5, 49). Due to this capacity, it has been tested as a vaccine against schistosomiasis and Crohn's disease. From animal models, it has been observed that administration of Sh28GST is as effective as *S. cercariae* in preventing tissue damage and inflammation (TNF, IL-1β, and IL-6 production) in experimental colitis. It was also found to induce Th2 polarization, characterized by local and systemic production of IL-13 and IL-5. An eosinophilic infiltration in colonic tissue was evidenced in GST-immunized mice, and eosinophil depletion was associated with a loss of the ameliorating effects observed for Sh28GST (49). This molecule was also tested in a curative model, observing therapeutic effects. Administration of P28GST after TNBS-colitis induction decreased gut inflammation, associated with Th1/Th17 response downregulation and the development of alternatively activated macrophages. In this review, the authors also demonstrated that only intact schistosome-derived P28GST was able to induce a significant reduction of the clinical score, in contrast to heat-inactivated P28GST that loses its enzymatic activity (85). Results from a multicenter, open-label, pilot Phase 2a study that evaluated the safety of P28GST administered to patients with mild Crohn's disease (CD) also indicated that this is

FIGURE 2 | Phylogenetic tree views of cytosolic helminth GSTs. The workflow was created in Phylogeny.fr (81). The amino acid sequences of the identified molecules were aligned using MUSCLE. The phylogenetic tree was constructed using PhyML. The editing was performed in the iTol platform (82).

a safe product and showed a promising effect on reducing disease severity (86).

Data from immunomodulation in humans can be extracted from clinical trials that tested this GST as a vaccine to prevent urinary schistosomiasis. Results from a Phase I study in 24 healthy volunteers who received Sh28GST with alum indicated that immunization with this antigen is mostly safe, with few cases of mild site-infection reactions. In a subset of patients ($n = 8$), whose humoral and cytokine response to Sh28GST-Alum was investigated, it was observed that immunization induced

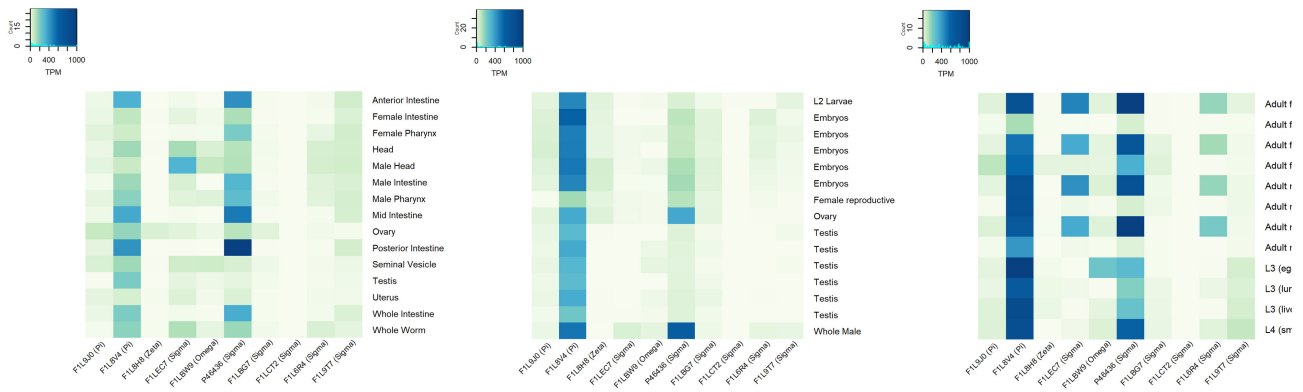

FIGURE 3 | Gene expression profile of GSTs from *Ascaris suum* based on public RNAseq data from experiments SRP013609 **(left)**, SRP005511 **(middle)**, and SRP010159 **(left)**. RNA expression levels in transcripts per million (TPM) in different tissues and lifecycle stages are showed (data from public RNA sequencing studies downloaded from parasite.wormbase.org).

IgG1, IgG2, IgG3, and IgG4 antibodies, in contrast to IgE whose titers did not significantly raise in comparison with baseline. Production of IL-5 and IL-13 was significantly induced after two administrations of rSh28GST in six out of eight vaccinated adults (5). In the Phase III study of this vaccine, 250 children, living in a hyperendemic area of Senegal, were randomized to receive rSh28GST or placebo. The vaccine did not show efficacy to prevent cases of urinary schistosomiasis. The anti-Sh28GST response was characterized by elevated levels of specific IgG1, IgG2, and IgG4 antibodies. In this population, vaccination did increase the specific IgE antibody response with 36.8% positive children in the active group. No cases of anaphylaxis or another systemic reaction after vaccination were reported; however, it is important to highlight that in contexts of natural exposure to helminths, helminths vaccines inducing IgE may induce anaphylaxis, as it was reported for a hookworm vaccine (87).

CROSS-REACTIVITY

Due to their potential clinical impact, GSTs from environmental sources, such as cockroaches and HDM, are allergens that could be included in component-resolved diagnosis platforms; it will then be necessary to define the clinical impact of cross-reactivity among them. However, there are few studies regarding this topic, probably because the complete repertoire of isoenzymes has not been elucidated in the species of interest. Mueller et al. analyzed *in silico* the potential of IgE cross-reactivity among some of the most studied allergenic GSTs and concluded that the possibility of this phenomenon is low (24). Although Bla g 5 and Asc l 13 are sigma-GSTs, they share scarce surface conservation, not enough to have predicted common epitopes. As expected, less similarity is found among mu- and sigma-GSTs (24). However, results from Santiago et al. suggest that cross-reactivity must be extensively evaluated even when it is not expected. For example, the piGST from *W. bancrofti* is cross-reactive with Bla g 5 despite only sharing 27% of sequence similarity. IgE, IgG, and IgG4 binding to Bla g 5 was inhibited to a significant extent (50–70%) in individual sera collected from cockroach-allergic patients. Modeling and sequence analysis indicated that both molecules may share a lineal epitope in their N-terminal domain (16).

Cockroach allergy has important clinical implications due to its association with emergency room admissions (35, 36). Besides HDM, sensitization to cockroach allergens is the next common risk factor associated with asthma in the tropics (37). In different tropical countries, cockroach sensitization is high, but this is not in agreement with the surprisingly low allergen levels found in house dust (38, 42), and it has been argued that this is because of cross-reactivity. Tropomyosins and GST families are candidates to mediate important clinical cross-reactivity. In a co-exposed population, we have observed a high correlation in IgE levels against helminth, cockroach, and HDM GSTs (23). However, in sera obtained from Bla g 5-positive patients living in the United States, and who are not expected to be exposed to tropical allergenic sources, such as Ascaris and *B. tropicalis*, IgE recognition of Asc l 13 or Blo t 8 was not observed. Moreover, IgE binding to Der p 8 was inhibited only by Der p 8, but not by Bla g 5, Blo t 8, or Asc s 13. These results support the idea that, at least in North America, IgE response to Bla g 5.0101 may reflect genuine sensitization to cockroaches and that cross-reactivity is not relevant. However, in the tropics, a deeper evaluation of cross-reactivity among GSTs might be needed. IgE cross-reactivity is present between Der p 8 and native *P. americana* GST (42) as well as between *B. tropicalis* and *A. suum* native GST (88). Identification of the correct isoforms mediating cross-reactivity is a further step to determine the clinical significance of this phenomenon.

DISCUSSION

Different GSTs from invertebrates have been identified as molecules with allergenic activity and potential clinical relevance. In addition, some of them are also frequent sensitizers in exposed populations. The identified allergens belong to different GST classes (sigma, mu, and delta); although with a similar

global structure, determined by key conserved amino acids and positions, there is low to moderate sequence conservation among them (**Figure 1**).

Several IgE-binding GSTs have been evaluated for their allergenic activity, being Bla g 5 the best characterized at the recombinant level, but more information on the complete repertoire of GSTs from *B. germanica*, observed in nature, is needed. Defining the allergenic activity will provide better information about the clinical impact of these IgE-binding molecules. In addition, extending the studies about their mechanisms of action for inducing allergic inflammation is necessary to improve our knowledge of allergy pathophysiology and to discover other options of allergy treatment. Their biological activity may be involved in Th2 polarization, which makes it interesting to explore other non-IgE mediated mechanisms that promote allergic responses (48).

Helminth-GSTs can induce different immune responses in the host; while *Schistosoma*-GSTs seem to induce a protective IgE response, the *Ascaris*-GSTs may induce allergic reactions (23). A possible explanation for this difference may be the whole context set up by the infection itself: routes of antigen entry, type 2 adjuvants, and immunosuppressive molecular content (89). There is evidence from animal models that ascariasis could increase the IgE/Th2 responses to bystander antigens (90, 91). In humans, different studies have observed that Th2/IgE hyper-responsiveness induced by Ascaris infection can also boost the IgE responses to HDM allergens, besides its antigens (76, 92). In that sense, there is a possibility that the allergenic response against Ascaris-GST is promoted by other components of *Ascaris*. On the contrary, it is also possible that due to the nature of the infection, Schistosoma spp. has a more immunosuppressive net effect that reduces the chance of potential allergenic molecules, such as GSTs, to elicit IgE-mediated reactions leading to a topic manifestations. Thus far, most of the studies tend to support that schistosome infection reduces skin test responses and allergic responses (93, 94).

The current data suggest that cross-reactivity is not frequent among GST allergens and those detected seem to be non-clinically relevant; however, more research is needed to understand how molecules with a low degree of sequence homology exhibit cross-reactivity. It is expected that GSTs from different classes are not cross-reactive, but an important exception has been described (16). Since living species usually contain genes coding for more than one GST class, it is necessary to evaluate the complete repertoire of GST isoenzymes if cross-reactivity aims to be assessed. It is also important to elucidate the clinical significance of IgE-binding GSTs from helminths due to the risk of allergic reactions to vaccines or immunotherapy strategies that use GST as antigens.

AUTHOR CONTRIBUTIONS

JZ and LC: conceived this manuscript. AL and JZ: performed bioinformatic analysis and developed figures. All authors contributed to manuscript writing, reviewing, and editing.

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Component-Resolved Diagnosis of American Cockroach (*Periplaneta americana*) Allergy in Patients From Different Geographical Areas

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Background: Manifestation of respiratory allergy to American cockroach (*Periplaneta americana*) is prominent in the subtropical and tropical areas. However, co-existing perennial indoor inhalant allergies frequently compromise clinical diagnosis of cockroach allergy, and the analysis of sensitization pattern is limited by the lack of *Periplaneta* allergens widely available for component-resolved diagnostics (CRD).

Objective: To evaluate a collection of previously described recombinant *Periplaneta* allergens for CRD in cockroach allergy.

Methods: A panel of nine recombinant *Periplaneta* allergens (Per a 1–5, 7–10) was generated, purified, and subjected to physicochemical characterization by applying circular dichroism (CD) spectroscopy, dynamic light scattering (DLS), amino acid (AA) analysis, and mass spectrometry (MS). Patients ($n = 117$) from India, Korea, Venezuela, and Iran, reporting perennial respiratory indoor allergies with IgE sensitization to cockroach (*P. americana and/or Blattella germanica*), were included. The sensitization profile was monitored by the experimental ImmunoCAP testing.

Results: ImmunoCAP testing confirmed IgE sensitization to *Periplaneta* and/or *Blattella* extract in 98 of 117 patients ($r = 0.95$). Five out of 117 patients were sensitized to only one of the two cockroach species. Within the whole study group, the prevalence of sensitization to individual allergens varied from 4% (Per a 2) to 50% (Per a 9), with the highest IgE values to Per a 9. Patients from four countries displayed different sensitization profiles at which Per a 3 and Per a 9 were identified as major allergens in India and Korea. *Periplaneta*-derived lipocalin and myosin light chain were characterized as new minor allergens, designated as Per a 4 and Per a 8. *Periplaneta* extract showed higher

diagnostic sensitivity than all individual components combined, suggesting the existence of allergens yet to be discovered.

Conclusion: Utilization of a panel of purified *Periplaneta* allergens revealed highly heterogeneous sensitization patterns and allowed the classification of lipocalin and myosin light chain from *Periplaneta* as new minor allergens.

Keywords: cockroach allergy, American cockroach, *Blattella germanica*, component-resolved diagnosis, *Periplaneta americana* (Insecta)

INTRODUCTION

The most common indoor aeroallergens are house dust and storage mites, pet dander, mold, and cockroaches (1). German cockroach (*Blattella germanica*), American cockroach (*Periplaneta americana*), Oriental cockroach (*Blatta orientalis*), brown-banded cockroach (*Supella longipalpa*), and smoky brown cockroach (*Periplaneta fuliginosa*) have been reported to cause allergic asthma morbidity worldwide (2). Cockroach allergy is predominantly caused by *Blattella* and *Periplaneta* in temperate and (sub)tropical areas and is a global health problem due to the increasing infestation of cockroaches in human housing environments (3). Cockroach allergens are present in saliva, fecal particles, spermatophores, shredded skin, and desiccated remains of insect bodies (2). In studies including children and adults, presented to hospital, the prevalence of cockroach allergy ranges from 17 to 41% in the United States, and 60–80% of inner-city children with asthma were sensitized to cockroach. It has been suggested that exposure to cockroach allergens appears to have a greater effect on asthma morbidity than dust mite or pet allergens, in particular among inner-city children (2).

Allergic sensitization to cockroach is frequently investigated by skin testing using crude extracts and by the measurement of specific IgE to cockroach allergens (2). However, the usage of allergen extracts possesses some limitations. Commercial or self-prepared allergen extracts at most are standardized based on in-house assays, causing the lack of comparability between different manufacturers. Moreover, the lack of immune-dominant allergen(s) and the complex patterns of IgE responses to cockroach extracts have made it difficult to produce standardized cockroach allergen extracts (4). Therefore, one approach to overcome these drawbacks is the utilization of purified allergens for component-resolved diagnostics (CRD).

Whereas, allergic sensitization to *B. germanica* allergens has been investigated in detail, data referring to *Periplaneta* allergens are less available. However, the tested cockroach species frequently is not indicated in the respective reports. Of note, species-specific sensitization to *Periplaneta* and *Blattella* has been reported, and in one study, only 68% of *Blattella*-reactive patients showed sensitization to *Periplaneta* (5). *Vice versa*, in China, 25.7% of allergic patients were sensitized to *Periplaneta* allergens, whereas only 18.7% were sensitized to *Blattella* allergens (6), indicating allergenic differences between these cockroach species or different diagnostic sensitivity of the extracts used. Although homologous allergens have been described for *Periplaneta* and *Blattella*, the presence of different allergenic components between the species needs to be considered. So far, among 13 suggested *Periplaneta* allergens, 11 have been officially recognized by the WHO/IUIS Allergen Nomenclature Sub-Committee (7, 8). Per a 1 (25–45 kDa, midgut microvilli-like protein) (9–11), Per a 2 (42 kDa, aspartic protease-like protein) (12), Per a 3 (46–79 kDa, homologue of arylphorin and insect hemocyanin) (13, 14), Per a 5 (23 kDa, glutathione S-transferase) (15–17), Per a 6 (18 kDa, troponin C) (18), Per a 7 (33 kDa, tropomyosin) (19, 20), Per a 9 (43 kDa, arginine kinase) (21, 22), Per a 10 (28 kDa, serine protease) (23), Per a 11 (55 kDa, α-amylase) (24), Per a 12 (45 kDa, chitinase) (24), and Per a 13 (36 kDa, glyceraldehyde-3-phosphate dehydrogenase) (24). Although lipocalin (Acc.No. AY792948) (25, 26) and myosin light chain (Acc.No. JQ279816, unpublished) have been suggested as potential *Periplaneta* allergens and have been proposed as Per a 4 and Per a 8, they are less well-characterized.

So far, studies addressing the sensitizing capacity of *Periplaneta* allergens were mainly performed with selected allergens rather than by CRD using the panel of *Periplaneta* allergens. Of note, data on the prevalence of IgE binding to target allergens are highly variable between different studies (7). An ELISA-based CRD study conducted in Taiwan, enrolling *Blattella*-sensitized patients with persistent asthma and including recombinant Per a 1 through Per a 7 and Per a 9, revealed a reactivity with Per a 2 and Per a 9 to be associated with severe asthma and allergic rhinitis, respectively (12). Taken together, the reports on the contribution of individual *Periplaneta* allergens in cockroach allergy are not consistent. It is worth noting that study results on the clinical significance of individual *Periplaneta* allergens may vary substantially depending on the quality of purified allergens used for antibody detection, patient inclusion criteria, co-exposure to cross-reactive allergens from other sources, and the geographical area where the study is conducted.

The present study aimed to investigate the molecular sensitization profile in a substantial number of cockroach-sensitized patients recruited in four different countries, all with perennial respiratory indoor allergy. For the purpose of CRD, a set of nine recombinant *Periplaneta* allergens were produced and characterized by uniform methods.

MATERIALS AND METHODS

Patients and Patient's Sera

For CRD, patients ($n = 117$) reporting perennial respiratory indoor allergy with confirmed IgE sensitization to *P. americana* and/or *B. germanica* were included in the study. Patients'

sensitization to cockroach was monitored by IgE-ELISA (India, $n = 35$) and IgE-immunoblotting (Iran, $n = 30$) using the self-prepared *Periplaneta* extract, by SPT using *Blattella* extract (ALK, Round Rock, TX, United States) (Venezuela, $n = 25$), and *Blattella* extract ImmunoCAP (i6, Thermo Fisher Scientific, Uppsala, Sweden) (Korea, $n = 27$), respectively. Screening of *Periplaneta* allergen expression and purification was done using sera from cockroach-allergic patients (Germany, $n = 1$; Plasmalab, Everett, WA, United States, $n = 6$). Ethical approval was obtained from IHEC (no. CLP 0019, CSIR-IGIB) and Isfahan University of Medical Sciences and Health Services (no. 295264).

Preparation of *P. americana* Extract

Periplaneta americana extract was produced as described in the **Supplementary Material**. In brief, proteins were extracted from lyophilized powder of defatted whole-body *Periplaneta* cockroaches using phosphate-buffered saline (PBS), and the protein fractions were gained after subsequent centrifugation and filtration steps.

Generation and Physicochemical Characterization of Recombinant *Periplaneta* Allergens

Detailed steps for the preparation of recombinant Per a allergens are depicted in the **Supplementary Material**. For cDNA cloning, published GenBank amino acid (AA) sequences were used as template, and signal peptides were excluded from the sequence. Five out of the nine proteins were produced as non-tagged proteins. Per a 2 and Per a 3 contain a C-terminal His_{tag}, whereas Per a 5 and Per a 10 comprise a N-terminal His_{tag} for purification. All proteins were expressed in *Escherichia coli* and purified by multiple chromatographic steps. Final protein concentration and purity was checked by bicinchoninic acid (BCA) (Sigma-Aldrich, United States), amino acid analysis (AAA), and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Additionally, mass spectrometry (MS) (intact masses and tryptic digestion) analysis was performed to confirm the identity of recombinant proteins. To determine the structural integrity and the aggregation status of the proteins, circular dichroism (CD), and dynamic light scattering (DLS) were carried out. Recombinant expression of individual allergens and analytical methods are described in the **Supplementary Material**.

IgE Immunoblot and CRD

Immunoblotting was performed to (1) demonstrate IgE sensitization of Iranian patients recruited by clinical history (not shown) and (2) to assess the IgE sensitization pattern for selected patients lacking detectable IgE binding to purified *Periplaneta* allergens. Briefly, *P. americana* extract (50 μg/cm) was applied to SDS–PAGE, transferred to nitrocellulose membrane (0.2 μm, Amersham Protean, GE Healthcare, Freiburg, Germany) by semi-dry blotting, and visualized by Ponceau S staining (Sigma-Aldrich, Munich, Germany). After blocking with 0.3% Tween-20 in Tris-buffered saline (TBS, 50 mM Tris, 150 mM NaCl, pH 7.4), the membrane was incubated overnight with patient's serum (500 μl/strip, diluted 1:10 in TBS, 0.05% Tween-20, 0.1% BSA), and bound IgE was detected as described elsewhere (27).

Specific IgE values to *Periplaneta* (i206) and *Blattella* extract (i6) were determined by ImmunoCAP tests (Thermo Fisher Scientific) and to single recombinant *Periplaneta* allergens by experimental ImmunoCAP tests as previously described (28). Assay background of each test was assessed and cutoff level for positivity adapted as required (0.35 kU_A/L for Per a 2, Per a 5, and Per a 10 and 0.10 kU_A/L for all other allergens).

RESULTS

Generation of Recombinant *Periplaneta* Allergens

All recombinant *Periplaneta* allergens were expressed in *E. coli* as non-fusion as well as N- or C-terminal His_{tag} proteins. After multistep chromatography, purified proteins were characterized by uniform physicochemical methods as indicated in **Supplementary Table 1**. Purity and apparent molecular weight of all proteins were assessed by SDS–PAGE performed under reducing conditions (**Figure 1**).

The identity of all nine recombinant *Periplaneta* proteins included in the study was confirmed by MS analysis, by either intact mass analysis or in-solution/in-gel digestion and AA analysis. The identity of Per a 6 could not be confirmed, and it was therefore excluded from the study. Of note, the MS analysis revealed that a high proportion of Per a 8 had a truncation at the N-terminus of the protein, with only 10% being present as the full-length protein (**Supplementary Table 1**). The truncation observed for Per a 8 did not affect the secondary structure of the protein. CD spectroscopy confirmed that most of the allergens show structural integrity or were at least partially folded (**Supplementary Table 1**). Analysis by DLS revealed that some recombinant allergens tended to partially aggregate; however Per a 4, Per a 8, and Per a 9 were determined as mostly monomeric proteins.

IgE Sensitization to *Periplaneta* and *Blattella* Allergens

Periplaneta- and *Blattella*-specific IgE was analyzed by ImmunoCAP testing in all 117 serum samples (**Figure 2**),

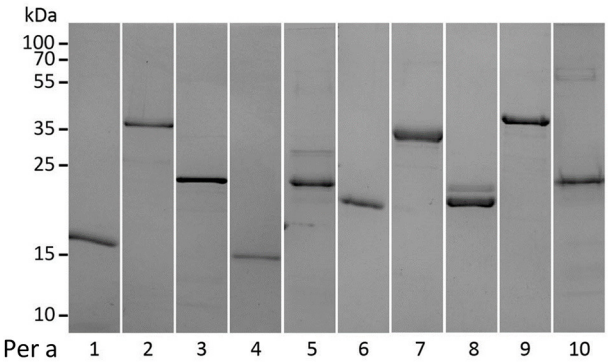

FIGURE 1 | SDS–PAGE and Coomassie staining of recombinant *Periplaneta* allergens, Per a 1 to Per a 10 (1-10), under reducing conditions.

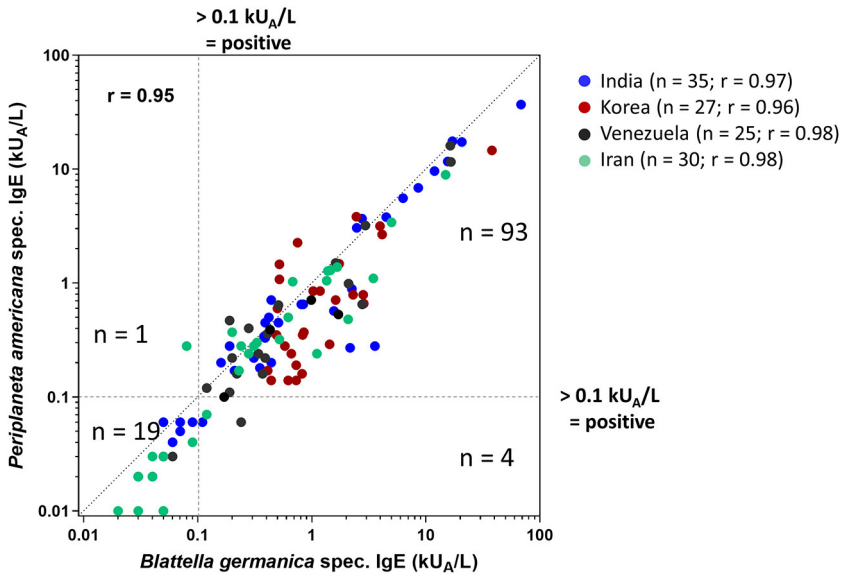

FIGURE 2 | Comparison of *Periplaneta* (i206)- and *Blattella* (i6)-specific IgE values in 117 patients (India, $n = 35$; Korea, $n = 27$; Venezuela, $n = 25$; Iran, $n = 30$) reporting cockroach allergy by ImmunoCAP testing (cutoff 0.1 kU$_A$/L); r-values are indicated.

showing an overall interspecies correlation of $r = 0.95$ and $r = 0.96$–0.98 for individual patient collectives. *In vitro* IgE reactivity to *Blattella* and *Periplaneta* was confirmed in 83% (97/117) and 81% (95/117) of the patients, respectively. Four subjects were sensitized to *Blattella* but not to *Periplaneta*, and one was sensitized to *Periplaneta* but not to *Blattella*. In total, 98 of 117 patients reacted to one or both of the cockroach species (**Figure 2**). Although patients were preselected by sensitization to cockroach by ELISA, SPT, and immunoblotting, 19/117 sera (comprising 6/35 from India, 3/25 from Venezuela, 10/30 from Iran) were tested negative to both species by ImmunoCAP.

Component-resolved Diagnosis of *Periplaneta* Allergy by ImmunoCAP Testing

In total, nine recombinant *Periplaneta* allergens (Per a 1 to Per a 10, except Per a 6) were included for CRD by experimental ImmunoCAP testing. Since the preparation of Per a 2, Per a 5, and Per a 10 displayed a tendency of unspecific IgE binding, the threshold for test positivity was set to 0.35 kU$_A$/L for these allergens (**Figure 3B**). Other *Periplaneta* allergens were evaluated with a cutoff of 0.1 kU$_A$/L (**Figure 3A**). Although no major allergen could be identified, Per a 3 and Per a 9 appeared as the most important in this study. The frequencies of sensitization to the different allergens in the entire study population were as follows: Per a 1: 16% (16/98), Per a 3: 41% (40/98), Per a 4: 18% (18/98), Per a 7: 28% (27/98), Per a 8: 24% (23/98), and Per a 9: 50% (49/98) (**Figure 3A**). Per a 4 and Per a 8 were characterized as minor allergens in comparison with other *Periplaneta* allergens. The frequency of IgE recognition of Per a 2, Per a 5, and Per a 10 was 4, 19, and 21%, respectively (**Figure 3B**).

The sensitization pattern within each patient group was diverse and not comparable between the different patient collectives (**Figure 4**). Analysis of individual patient groups from different geographical areas revealed the highest prevalence of IgE reactivity to Per a 3 (59 and 67%) and Per a 9 (57 and 67%) in Indian and Korean patients. Per a 9 was the most prominent (45%) allergen also among the Iranian patients, whereas patients from Venezuela reacted predominantly to Per a 7 (31%) (**Figure 4**). Per a 4 and Per a 8 were identified as minor allergens in patients from almost all investigated geographies. However, the prevalence of sensitization to these components was the lowest among subjects from Venezuela. Patients from India and Korea showed low frequencies of IgE reactivity to Per a 2, Per a 5, and Per a 10 (**Figure 4**) (India: 3% to Per a 2, 28% each to Per a 5 and 10; Korea: 11% to Per a 2, 44% to Per a 5 and 48% to Per a 10). Notably, none of the Venezuelan or Iranian patients were sensitized to any of these three proteins (**Figure 4**).

Of the 95 *Periplaneta* ImmunoCAP-positive patients, 28 (29%) did not react with any of the purified allergens tested (**Supplementary Table 2**). This discrepancy was most prominent in serum samples from Iran 9/19 (47%), but less frequent in samples from India 6/28 (21%), Korea 7/27 (26%), and Venezuela 6/21 (29%). It is tempting to speculate that these patients are sensitized to other *Periplaneta* proteins or isoforms not reflected by the present CRD panel. In contrast, only 4% (5/117) of the patients showed IgE sensitization (with IgE levels close above the cutoff) to one or more of the purified allergen(s) despite a negative test result with *Blattella* and *Periplaneta* ImmunoCAP.

IgE Immune Reactivity of Periplaneta Extract

Sera of cockroach-sensitized patients ($n = 7$) from Venezuela either lacking any IgE reactivity (VR03, VR06, VR10, VR20) or showing very low IgE values of 0.12 kU$_A$/L (VR07, VR12)

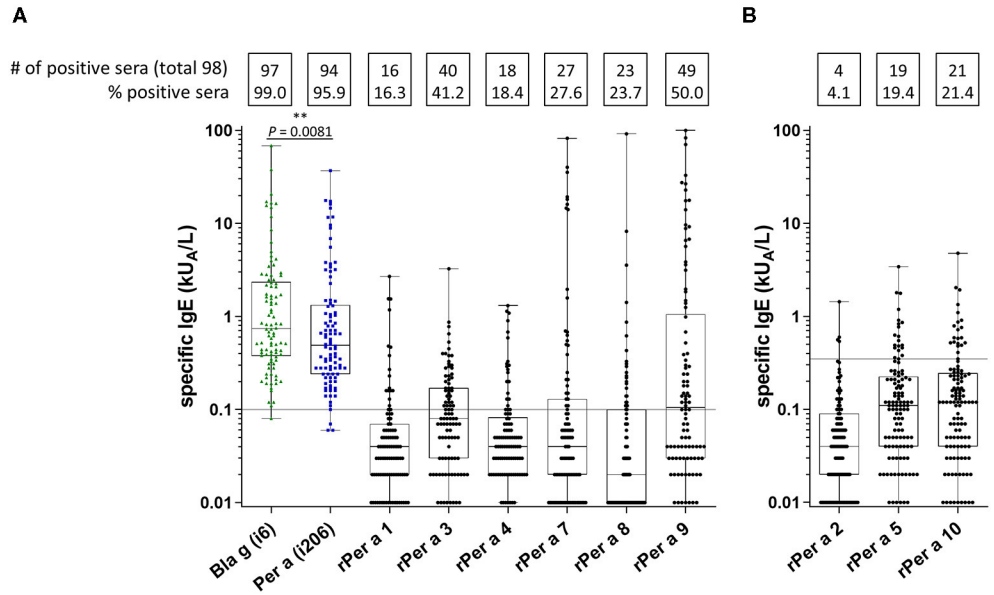

FIGURE 3 | CRD of cockroach allergy by experimental ImmunoCAP testing including cockroach-allergic patients preselected by positive *Periplaneta*- or *Blattella*-specific ImmunoCAP values ($n = 98$). Specific IgE values for *Periplaneta* and *Blattella* extracts and Per a 1, Per a 3, Per a 4, Per a 7, Per a 8, and Per a 9 (cutoff 0.1 kU_A/L) **(A)**, as well as for Per a 2, Per a 5, and Per a 10 (cutoff 0.35 kU_A/L) **(B)** are depicted. (paired *t*-test).

	Bla g (i6)	Per a (i206)	rPer a 1	rPer a 2*	rPer a 3	rPer a 4	rPer a 5*	rPer a 7	rPer a 8	rPer a 9	rPer a 10*
total (n = 98)	97 (99.0%)	94 (95.9%)	16 (16.3%)	4 (4.1%)	40 (41.2%)	18 (18.4%)	29 (20.4%)	27 (27.6%)	23 (23.7%)	49 (50.0%)	21 (21.4%)
Indian (n = 29)	29 (100%)	28 (96.6%)	3 (10.3%)	1 (3.4%)	17 (58.6%)	7 (24.1%)	8 (27.6%)	7 (24.1%)	8 (27.6%)	17 (58.6%)	8 (27.6%)
Korean (n = 27)	27 (100%)	27 (100%)	7 (25.9%)	3 (11.1%)	18 (66.7%)	7 (25.9%)	12 (44.4%)	9 (33.3%)	7 (25.9%)	18 (66.7%)	13 (48.1%)
Venezuela (n = 22)	22 (100%)	20 (90.9%)	4 (18.2%)	0 (0.0%)	2 (9.1%)	1 (4.5%)	0 (0.0%)	7 (31.8%)	3 (13.6%)	5 (22.7%)	0 (0.0%)
Iran (n = 20)	19 (95.0%)	19 (95.0%)	2 (10.0%)	0 (0.0%)	3 (15.0%)	3 (15.0%)	0 (0.0%)	4 (20.0%)	5 (25.0%)	9 (45.0%)	0 (0.0%)

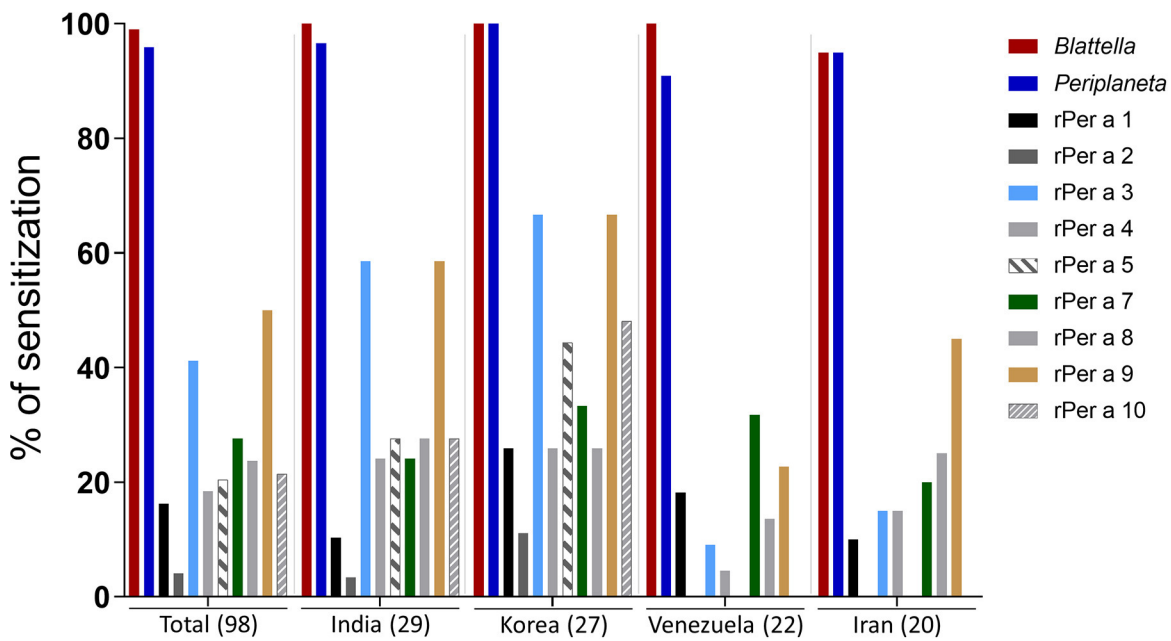

FIGURE 4 | Comparison of IgE antibody levels to *Periplaneta* (i206), *Blattella* (i6), and purified recombinant *Periplaneta* allergens in respective patient collectives from India ($n = 29$), Korea ($n = 27$), Venezuela ($n = 22$), and Iran ($n = 20$). Prevalence (%) of IgE sensitization to Per a 1, Per a 3, Per a 4, Per a 7, Per a 8, and Per a 9 (cutoff of 0.1 kU_A/L) and Per a 2*, Per a 5*, and Per a 10* (cutoff of 0.35 kU_A/L) are shown.

to purified allergens were further analyzed by immunoblotting using *Periplaneta* extract (**Figure 5**). Two sera (Sal and PL6) were applied as positive controls. IgE binding to putative novel and yet not identified *Periplaneta* proteins was demonstrated for six out of seven sera, showing IgE binding to distinct high molecular weight (HMW) proteins and proteins with an apparent molecular weight of 16 kDa (VR3, VR12), 22 kDa (VR12), and 40 kDa (VR6, VR9, and VR20) not verified so far.

However, the accumulation of both proteins, lipocalin and myosin light chain, in *Periplaneta* extract was confirmed by MS/MS analysis. Peptides derived from both allergens showed a sequence coverage of 15 and 29% (for Per a 4) and 34 and 59% (for Per a 8), in two extracts analyzed independently (**Supplementary Figure 3**).

DISCUSSION

Cockroaches are an important source of indoor aeroallergens. However, allergens from American cockroach (*P. americana*) are less investigated in comparison with German cockroach (*B. germanica*) allergens, and reports on the involvement of individual *Periplaneta* allergens in allergy related morbidity are inconsistent. Notably, clinically relevant sensitization to *Blattella* across Europe was reported in the GA^{2}LEN skin test study (29). In addition, sensitization to different cockroach species has been reported in South Italy (30), demonstrating positive RAST results to *Periplaneta* in four of 15 cockroaches (mixed species) SPT-positive patients (31). However, in Italian children, sensitization was substantially higher to *Periplaneta* than to *Blattella* (32). So far, the molecular sensitization profile to *Periplaneta* allergens was not addressed in these studies, except one study describing reactivity to Per a 7 (tropomyosin) in 41% of *Periplaneta*-sensitized indoor allergic patients from Marseille (19). All these studies implicate that cockroach allergen exposure and sensitization are a global health problem that will likely increase along with global warming. This prompted us to investigate the involvement of *Periplaneta* allergens in the sensitization profile to cockroach.

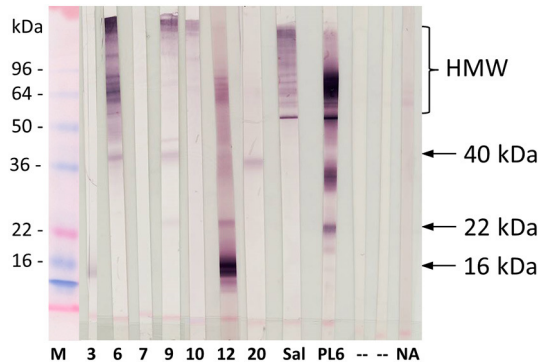

FIGURE 5 | IgE-reactivity pattern of cockroach-reactive patients from Venezuela ($n = 7$, VR3, VR6, VR7, VR9, VR10, VR12, VR20) showing negative ImmunoCAP results for recombinant *Periplaneta* allergens, Germany (Sal), and pooled sera from cockroach-sensitized patients (PL6) with *Periplaneta* extract. M, marker; P, Ponceau S, staining; NA, non-atopic control; "-", secondary antibody control. Arrows indicate the putative yet not identified allergens.

In the present study, using different patient collectives, a substantial panel of recombinant *Periplaneta* allergens, including yet less well-characterized allergens Per a 4 and Per a 8, was utilized for CRD employing a uniform analytical methodology. The identity of all purified *Periplaneta* allergens used in the study was confirmed by MS. We attempted to produce Per a 6 but were unable to confirm its identity. Three known *Periplaneta* allergens Per a 11 to Per a 13 were not included in the study since they were not yet been identified at the time the study was initiated.

A total of 117 patients from four countries with subtropical or tropical climates, with perennial respiratory indoor allergy, and evidence of IgE sensitization to cockroach (evaluated by *in vitro* and *in vivo* methods), were included in the study. Of note, the limitation of the study is the lack of consistent inclusion criteria in terms of clinical cross-reactivity (co-sensitization with cross-reactive mite and insect allergens), and the application of different IgE detection methods in different cohorts, which can lead to a bias of the preselected patients. Cockroach-specific IgE was re-evaluated and confirmed in 81% and 83% of all sera by commercial ImmunoCAP testing, using *Periplaneta* or *Blattella* extracts, respectively. Whereas, sera from Korean patients were preselected by positive ImmunoCAP results, IgE sensitization of patients from Venezuela, India, and Iran was assessed by either skin testing, ELISA, or immunoblotting, showing a correlation with *Periplaneta*-positive ImmunoCAP testing between 63 and 80%. Discrepancy between results from ImmunoCAP and other test systems might be due to various applications of non-standardized and self-prepared extracts, as well as the different diagnostic sensitivity of each test system.

ImmunoCAP testing showed an almost similar diagnostic sensitivity for *Blattella* and *Periplaneta* extract and levels of IgE to the two cockroach species were strongly correlated ($r = 0.95$). Only one patient with low *Periplaneta*-specific IgE but negative *Blattella*-specific IgE values was detected. *Vice versa,* four patients with reactivity to *Blattella*, but without sensitization to *Periplaneta*, were identified. Finally, the diagnostic sensitivity using recombinant allergens was increased for five patients who did not react with any of the two extracts. The overall concordant results are likely explained by the presence of conserved and cross-reactive allergens in *Periplaneta* and *Blattella*, consistent with the official recognition of allergens from at least seven groups identified in both species. However, only partial IgE cross-reactivity between members of homologous groups has been reported (2). Slight variability of IgE-binding capacity between the species might be due to distinct expression levels of certain allergens in cockroaches, a variable presence of allergens in the applied extracts, or the abundance of species-specific allergens. In line with this, group 4 and 8 allergens were already denominated according to the IUIS allergen nomenclature database for *Blattella* but not for *Periplaneta*, whereas group 10 and 13 allergens have so far not been identified in *Blattella*. In the present study, we produced recombinant *Periplaneta* proteins homologous to Bla g 4 and Bla g 8 and included them in the panel of allergens used for CRD.

Component-resolved diagnostics was performed with patients ($n = 98$) reporting perennial respiratory indoor allergies and IgE sensitization to *Blattella* or *Periplaneta,* confirmed by ImmunoCAP testing as a uniform methodology across the entire

population. Results showed only 68% (67/98) of ImmunoCAP-positive patients to react with any of the nine recombinant *Periplaneta* allergens tested. However, a study addressing CRD of *Blattella* allergy suggested a panel of only four allergens (Bla g 1, Bla g 2, Bla g 4, and Bla g 5) to be sufficient to identify 95% of cockroach-allergic patients in the United States (33). In another study, all patients with airway allergy and positive *Blattella* ImmunoCAP values were reported to react with at least one of Per a 1 to 7 and Per a 9, tested by ELISA (12). In contrast, our study is in agreement with other reports demonstrating that a set of several recombinant *Blattella* allergens fall short of reflecting the IgE reactivity of corresponding natural *Blattella* allergen extract (34, 35). By applying a panel of allergens, only 49% and 62% of cockroach-allergic patients were tested positive either by skin testing (35) or by *in vitro* assays (34), respectively. Both studies are in accordance with our present results showing a higher diagnostic sensitivity of the natural extract in comparison with the applied recombinant allergens. The higher diagnostic value of cockroach extract in comparison with the panel of purified allergens can be explained by missing allergens which need to be included in CRD, structural modification of recombinant allergens affecting the IgE reactivity (e.g., mediated by posttranslational modifications, or partial aggregation of recombinants). Furthermore, the presence of IgE-reactive natural variants/isoforms that are not reflected by the selected recombinant allergens could play a role in the lower diagnostic sensitivity of the CRD.

The present study demonstrated a heterogeneous pattern of IgE sensitization to *Periplaneta* allergens in different patient collectives. The IgE-reactivity profiles could be in part due to the exposure and IgE responses to abundant cross-reactive homologous allergens. For all *Periplaneta* allergens except Per a 1, homologous groups and potential cross-reactive allergens have been described among invertebrates other than cockroaches (1). In agreement with a previous notion by Pomes et al. (1), we did not observe any dominant and thus major *Periplaneta* allergen considering the whole study collective. Importantly, the analysis of the individual geographical different cohort revealed Per a 3 and Per a 9 as major allergens (with IgE prevalence $\geq$50% in the respective patient group) in India and Korea. This, together with the occurrence of extract-positive subjects without IgE to any of the components included in this study, indicates the need to identify and evaluate additional *Periplaneta* allergens in each cohort.

In the present study, lipocalin and myosin light chain were classified for the first time as minor *Periplaneta* allergens in all investigated cohorts with an overall frequency of IgE sensitization of 18 and 24%, respectively. Both proteins were accepted by the WHO/IUIS Allergen Nomenclature Sub-Committee as Per a 4 and Per a 8. Sensitization to recombinant Per a 4 was most prevalent in patients from Korea (26%), and sensitization to recombinant Per a 8 was most prevalent in Indian patients (28%). Whether the IgE-binding capacity of recombinant allergens is reflected by the natural counterparts remains open. However, our study provided evidence that the prominence of allergens is different in the respective patient collective. The results are in agreement with a limited number of studies describing molecular sensitization profiles to *Periplaneta* allergens and showing a heterogeneous clinical significance of single allergens. In line with this, the prevalence of IgE sensitization of 5–100% for Per a 1, 63% for Per a 2, 26–95% for Per a 3, 30–70% for Per a 5 (16), 13–54% for Per a 7, 80–100% for Per a 9, 82% for Per a 10, 83% for Per a 11, and 64% for Per a 12 has been reported (7). A retrospective study of 118 cockroach-sensitized subjects from inner-city environments in the United States revealed that only 13% were sensitized to Per a 7 (34). In contrast, Per a 7 was found to be the dominant allergen among 55 cockroach-allergic patients in Brazil, with a prevalence of IgE reactivity of 42% (35).

Our data suggested the involvement of yet unidentified *Periplaneta* allergens (including isoforms and variants) and/or a potential important role of Per a 6, Per a 11 to Per a 13, which were not tested in the current study. Moreover, 2D-immunoblotting and subsequent MS analysis using pooled sera, not reactive to any of the tested recombinant Per a allergens, revealed IgE binding to 12- and 16-kDa proteins, in addition to a 40-kDa band which might correspond to Per a 11 to Per a 13 (data not shown). In addition, the role of protein glycosylation in the immunogenicity of cockroach allergy (2) needs to be considered. Insects express immunogenic core α-1,3 carbohydrate structures with additional −1,6 fucosylation (36), and glycan modification of *Blattella* allergens has been described (37, 38). Although reports on cross-reactive carbohydrate determinants (CCD) of cockroach allergens are limited, and routine diagnosis lacks insect-specific glycan moieties, it is tempting to speculate that a substantial number of cockroach-sensitized patients are reactive to glycan structures which in part may explain our observed gap in diagnostic sensitivity between natural cockroach extracts and a panel of recombinant, non-glycosylated allergens.

In summary, we show that levels of IgE antibodies to *Periplaneta* and *Blattella* are strongly correlating and that natural *Periplaneta* extract displays higher diagnostic sensitivity than a panel of nine recombinant *Periplaneta* allergens. For reasons that are elusive, patient collectives from different countries showed heterogeneous sensitization profiles. Notably, major allergens could be identified only for individual cohorts, while Per a 4 and Per a 8 were identified as minor allergens. Further improvement of *Periplaneta* CRD requires exploration of the role of isoforms, glycan determinants, and additional yet unknown allergens, which are likely classified already in other insects.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

Ethical approval was obtained from IHEC (no. CLP 0019, CSIR-IGIB) and Isfahan university of Medical Sciences and Health

services (no. 295264). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JS, RR, RS, SSa, FT, J-WP, and NA recruited and characterized the patients. Recombinant Per a 1, Per a 2, and Pera 3 were prepared and characterized by SE and IP. Per a 4, Per a 7, Per a 8, and Per a 9 by AW and AJ. Per a 5, Per a 6, and Per a 10 by SSh. Protein and MS analysis were performed by BS, BK, SW, GG and PB. Experimental ImmunoCAPs were established and performed by JL and FFü. NA, FFe, and SV were responsible for the study. GG, AW, and SSc coordinated the study and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REVIEW
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Seafood Allergy in Asia: Geographical Specificity and Beyond

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Asian countries have unique ways of food processing and dietary habits that may explain the observed differences in the prevalence, natural history, epidemiology and sensitization pattern of food allergic diseases when compared to western countries. Per capita consumption of seafood, including fish and shellfish, is well above the global average for many Asian countries because of their coastal geographical location and rich seafood supply. The wide availability and high abundance of seafood in Asian countries have shaped a diverse way of processing and eating this major food group. Such unique features have significant impact on the sensitization profile and allergenicity of Asians to fish and shellfish. For example, fish and shellfish are eaten raw in some countries that may promote sensitization to heat-labile allergens not otherwise seen in other regions. Fermented fish sauce is commonly used as a condiment in some countries which may promote fish sensitization. Shrimp head and shrimp roe are regarded as delicacies in some countries, but their allergen profiles are yet to be characterized. Freshwater fish and shellfish are a common food source in many Asian countries but the allergenicity of many such species remains unknown. In this review, we discuss factors that may contribute to differences in molecular profile and sensitization pattern for fish and shellfish that are observed in Asian populations and revisit the current status of seafood allergy in this part of the world. Acknowledging the similarities and differences of seafood allergy patterns between Asian and western populations can help us refine a better strategy for diagnosing and managing seafood allergy.

Keywords: prevalence, tropomyosin, parvalbumin, Crustacea, mollusk, fish

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INTRODUCTION

Food allergy is a common allergic disease that affects up to 10% of the population, and its prevalence has been increasing over the past decades (1, 2). While the "big eight" food groups account for 90% of reported allergic reactions (3), the most common triggers of food allergic reactions in a particular geographical location are largely influenced by the feeding pattern, dietary habit and food availability (4). Per capita consumption of seafood, including fish and shellfish, is much higher in Asia when compared to the global average. Asian countries consumed two-thirds of global seafood production (5). In light of this, it is not surprising that seafood allergy is of high importance in Asia where its prevalence was up to 7.7% in some countries (6). Furthermore, Asian countries have distinct ways of seafood processing and dietary habits, which may account for substantial differences observed in the natural history and sensitization profile of seafood

allergic subjects when compared to western countries. For example, fish is introduced early into the infants' diet that parallels the younger age at onset of fish allergy in Asian children (7). Seafood is often consumed raw in Asian cuisines, and this eating practice can promote sensitization to heat-sensitive seafood allergens which is not reported in western countries. Besides, Asians consume some seafood species that are not being consumed in other parts of the world. With the advancement of molecular allergology, we are now able to examine allergen components in depth. Emerging studies have reported the occurrence of monosensitization to particular seafood species in this region. In this review, we discuss seafood allergy from the Asian perspective. Acknowledging important differences between East and West and unique features of seafood allergy in Asia would provide important insight on the clinical management of seafood allergy.

PREVALENCE AND NATURAL HISTORY OF SEAFOOD ALLERGY IN ASIA

Prevalence of Shellfish Allergy in Asian Countries

Shellfish is one of the most common food allergen sources in Asia (**Table 1**), and the leading cause of food allergy in Taiwan (18, 19), Thailand (20), Singapore (21), Vietnam (9, 12) and Hong Kong (22). In a survey in which IgE sensitization and skin prick tests were used to determine food allergy prevalence, shrimp was found to be the most commonly sensitized food allergen source in Hong Kong, China and India (8). Up to 13.1 and 10.3% of the Chinese and Indian populations had shrimp-specific IgE (sIgE) that exceeded 0.70 kUA/L, while over 2% of the Hong Kong population was positive to shrimp by skin prick test. On the other hand, shellfish allergy is less common in East Asia where shellfish only accounted for 3.4% of all reported food allergy cases in Japan (23) and 0.87% of parent-reported shellfish allergy in South Korea (13). The prevalence of shellfish allergy was affected by environmental exposures, dietary habits and cross-sensitization with other arthropods such as dust mite or cockroaches (24, 25).

Prevalence of Fish Allergy in Asian Countries

Fish allergy is less common in Asia than shellfish allergy, but its prevalence is still considerably high (**Table 1**). Fish allergy affected 1.55–1.60% of Vietnamese, which was second to shellfish as the most common type of food allergen source (9, 12). The prevalence of self-reported fish allergy was 2.29% in Philippines where fish is part of the weaning diet and fish sauce widely used as a condiment (26). In contrary, parent-reported fish allergy affected <1.5% of other Asian populations (11, 13, 22, 26). The rates of IgE sensitization to fish were even lower, when none of the 10,681 subjects in China was positive for fish-specific IgE and only 1.2% of subjects in Hong Kong were sensitized to fish (27). Nevertheless, this study only selected cod as the benchmark for sIgE, when this fish species is not popular in many Asia populations. Thus, results from this study probably underestimated the prevalence of fish allergy in Asia.

Natural History of Shellfish Allergy in Asia

Seafood allergy is generally believed to be persistent throughout life despite limited published evidence on its natural history (28). Shellfish allergy had late onset at a median age of 25 years old in Canadians (29), whereas shellfish allergy started much earlier in the Asian population. While shellfish allergy affected 0.1% of children aged 0–5 years in the United states (30), IgE sensitization to shellfish was as high as 10.6% among Singaporean children younger than 3 years of age (31). It was noteworthy that shellfish was the most frequent cause of food allergy in several studies that involved school-age or pre-school Asian children (9, 12, 18–22), which was probably attributed to the early introduction of shellfish in the Asian diet. Another possible reason is that exposure to other arthropods like dust mite or cockroach in tropical or subtropical areas of Asia may increase the shellfish allergy through cross-reactive allergens. Shellfish allergy also tends to persist longer as shellfish was reported to be the leading cause of food-induced anaphylaxis in older children and adults in Singapore (32), Hong Kong (33) and Thailand (34). Nevertheless, a Thai study found that 46% of shrimp-allergic patients were able to tolerate shrimp 10 years later, suggesting that some individuals could outgrow shrimp allergy (35). In addition, Ayuso et al. reported greater epitope recognition for shrimp in children than adults, supporting that shrimp sensitization could decrease with age (36). It remains unclear at this stage whether this is a general observation or is limited to some populations.

Natural History of Fish Allergy in Asia

Similar to shellfish allergy, it is generally believed that fish allergy persists throughout life although the relevant evidence is limited. One study suggested that 65.5% of fish-sensitized infants maintained their sensitization at school age (37). Another study reported that the prevalence of fish allergy was slightly higher in American adults (0.9%) than children (0.6%) (38). The same study also found that nearly 40% of adults having fish allergy started to suffer from this allergy in adulthood. Similar findings were reported in Philippines (26). Alternatively, a small study reported that sIgE levels and skin prick test positivity of fish-allergic patients diminished over a 9-year period, and some subjects were able to tolerate fish with lower allergenicity such as tuna and swordfish (39). Longitudinal studies are required to elucidate the natural history of fish allergy. It is also noteworthy that allergenicity of different fish species could vary significantly, and it is important to distinguish between fish-allergic patients with partial tolerance and true resolution of their fish allergy.

Shellfish and Fish Allergen Sources and Allergens

Molecular Characteristics of Shellfish Allergens

The filamentous muscle protein tropomyosin was first identified as a shrimp allergen in 1993 (40), which was subsequently reported to be a pan-allergen among invertebrates including crustaceans, arachnids, insects and mollusks (41). Tropomyosin is a heat-stable allergen that can withstand high temperature, food processing methods including ultrasound and gamma irradiation, and gastric digestion (42). Arginine kinase was identified as another pan-allergen but, in contrast to

TABLE 1 | Population-based survey on the prevalence of seafood allergy in Asian countries over the past 10 years.

References	Location	Sample size	Age range (Year)	Methodology	Shellfish (rank)	Fish (rank)
Li et al. (8)	Hong Kong	6,194	6–11	sIgE > 0.7 kUA/L	4.7% (3rd)	1.2% (21st)
				SPT	2.43% (1st)	0.22% (4th)
				sIgE + symptoms	1.05% (1st)	0.2% (2nd)
	Guangzhou	5,542	6–11	sIgE > 0.7 kUA/L	2% (2nd)	0% (/)
				SPT	1.14% (1st)	0% (/)
				sIgE + symptoms	0.18% (1st)	0% (/)
	Shaoguan	5,139	6–11	sIgE > 0.7 kUA/L	13.1% (1st)	0% (/)
				SPT	4.53% (1st)	0.04% (10th)
				sIgE + symptoms	0.65% (1st)	0% (/)
	Bengaluru/Mysore	5,677	6–11	sIgE > 0.7 kUA/L	10.3% (1st)	0.4% (26th)
				sIgE + symptoms	0% (/)	0% (/)
Le et al. (9)	Vietnam	9,039	16–50	Self-reported	6.88% (1st)	3.71% (2nd)
				Doctor-diagnosed	2.95% (1st)	1.58% (2nd)
Dai et al. (10)	Wenzhou	4,151	3–6	Self-reported	2.46% (2nd)	1.35% (6th)
Ziyab et al. (11)	Kuwait	3,864	11–14	Questionnaire + Clinical history	1.3% (5th)	1.6% (3rd)
Le et al. (12)	Hue	4,443	2–6	Self-reported	5.22% (1st)	1.55% (2nd)
				Doctor-diagnosed	4.79% (1st)	1.37% (2nd)
	Tien Giang	4,177	2–6	Self-reported	4.29% (1st)	1.7% (4th)
				Doctor-diagnosed	2.8% (1st)	1.1% (4th)
Kim et al. (13)	Korea	29,842	6–16	Self-reported	0.84% (2nd)	0.32% (3rd)
Park et al. (14)	Seoul	16,749	0–6	Self-reported	0.5% (5th)	0.4% (6th)
Dey et al. (15)	Kolkata	5,161	All	Self-reported	3.43% (/)	4.59% (/)
Lao-araya et al. (16)	Chiang Mai	452	3–7	Self-reported	3.32% (2nd)	1.1% (4th)
Ho et al. (17)	Hong Kong	7393	0–14	Self-reported	1.79% (1st)	0.19% (8th)
Wu et al. (18)	Taiwan	30,018	All	Self-reported	7.23% (1st)	1.32% (2nd)

tropomyosin, this heat-labile protein exhibited reduced IgE reactivity upon thermal and pH treatment (43). Myosin light chain, sarcoplasmic calcium-binding protein, troponin C, triosephosphate isomerase, and fatty acid-binding protein are other shellfish allergens being registered in the WHO/IUIS allergen database, although their physiochemical properties remain largely unknown (44, 45). Paramyosin was identified as an allergen in mollusk that have limited cross-reactivity with allergens in crustaceans (46).

Molecular Characteristics of Fish Allergens

The calcium-binding muscle protein parvalbumin was first identified as the major fish allergen more than 50 years ago (47). It is highly conserved across all bony fishes (48). Similar to tropomyosin, parvalbumin was resistant to thermal treatment and enzymic hydrolysis (47). Despite high homology across fish species, parvalbumin had different allergenicity in different fish species (48), and the allergenicity of individual fish species was also dependent on the quantity of parvalbumin present in the muscle (49). Collagen was identified as the second heat-stable fish allergen from muscle and skin of fishes (50, 51), but its allergenicity and characteristics were only studied in a small number of fish species. Two heat-labile muscle enzymes of fish, aldolase and enolase, were recently identified as allergens but their clinical relevance remained unclear (52). Tropomyosin was also registered as a fish allergen in the WHO/IUIS database, although its allergenicity has only been demonstrated in one fish species, the Mozambique tilapia. Apart from muscle proteins, fish roe was also reported to cause allergic reactions due to the presence of yolk protein vitellogenin (53).

Major Shrimp Allergens in the Asian Population

Seawater shrimps such as *Penaeus monodon* and *Litopenaeus vannamei* are the most widely distributed and marketed shrimps in the world, and their respective allergen profiles have been well-characterized (**Table 2**). Freshwater shrimps are also popularly consumed in Asia, including the giant freshwater prawn *Macrobrachium rosenbergii* in Thailand and Taiwan and the freshwater Siberian prawn *Exopalaemon modestus* in China, Korea and Taiwan. Tropomyosin was identified as the major shrimp allergen, and tropomyosins of freshwater and seawater shrimps shared over 95% sequence homology (54, 55). Arginine kinase and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), on the other hand, were identified as major shrimp allergens in *M. rosenbergii*, with arginine kinase being cross-reactive with *Gryllus bimaculatus* (field cricket) (56, 57). Interestingly, the oxygen-transport protein hemocyanin was found to be a species-specific allergen in *M. rosenbergii*. Hemocyanins of tiger shrimp and mud crab was unable to inhibit IgE binding to *M. rosenbergii* hemocyanin (58), while a Thai study reported isolated sensitivity to *M. rosenbergii* based on results of oral food challenges (59).

TABLE 2 | Summary of shellfish and fish allergens.

Allergen source	Allergen	Molecular weight (kDa)	Body part
Shellfish	Tropomyosin	34–38	Muscle
	Arginine kinase	40–45	
	Sarcoplasmic calcium-binding protein	20–25	
	Myosin light chain	17–20	
	Troponin C	21–21	
	Triosephosphate isomerase	28	
	Fatty acid-binding protein	15–20	
	Paramyosins	100	
	Myosin heavy chain	225	
	Pyruvate kinase 2	58	
	Filamin C	90	
	Hemocyanin	60–80	Cephalothorax
	Glyceraldehyde 3-phosphate dehydrogenase (GDPH)	37	Shell
	Vitellogenin	282	Ovary
	Ovarian peritrophin 1 precursor	30	
	β-actin	41–46	
	14-3-3 protein	27	
Fish	Parvalbumin	10–12	Muscle
	Enoloase	50	
	Aldolase	40	
	Collagen	130–140	
	Tropomyosin	32	
	Lipovitellin	150–160	Roe
	β'-component	16–35	

Major Crab Allergens in the Asian Population

Crab is another popular crustacean food item worldwide. In China, crab was the most common culprit for severe adverse food reactions caused by shellfish (8). Crab was also the most common food allergen source among Taiwanese schoolchildren while 16.2% of Singaporean adults had crab allergy (60). The more commonly consumed crabs in western countries include blue crab (*Callinectes sapidus*), snow crab (*Chionoecetes opilio*), Dungeness crab (*Cancer magister*), edible crab (*Cancer pagurus*) and king crab (*Paralithodes camtschaticus*) (21, 61), while Chinese mitten crab (*Eriocheir sinensis*), mud crabs (*Scylla paramamosain*, *Scylla serreta*, and *Scylla tranquebarica*), red crab (*Charybdis feriatus*) and blue crab (*Portunus pelagicus*) were more popularly consumed in Asia. The IgE-binding proteins of these latter crab species have been extensively studied (62–66). Unlike shrimps, both tropomyosin and arginine kinase are major allergens in crab (**Table 2**). Arginine kinase is a 41 kDa phosphagen kinase that plays an important role in cellular energy metabolism in invertebrates. Apart from these two major allergens, sarcoplasmic calcium-binding protein, triosephosphate isomerase, pyruvate kinase 2, myosin light chain I and filamin C were minor crab allergens (56, 67, 68). Jasim et al. (69) identified three common crab allergens including tropomyosin, arginine kinase and actin as well as a novel allergen hemocyanin from *S. transquebarica* that is commonly consumed in Malaysia.

Major Mollusk Allergens in the Asian Population

Besides crustaceans such as shrimp and crab, mollusks are popular food item in Asian countries. There is a common belief that mollusks offer health-promoting effects because of their rich vital nutrients and active secondary metabolites (70). Cross-reactivity between crustaceans and mollusks were well-recognized (42). Despite this, Suzuki et al. (46) identified a 100 kDa myofibrillar protein paramyosin in the disc abalone *Haliotis discus* as a novel allergen. This protein reacted with 16 of the 18 Japanese sera from allergic subjects being studied. They also reported IgE reactivity against paramyosins from turban shell, mussel, scallop, squid and octopus, which was subsequently found in the Asian rapa whelk *Rapana venosa* (71). Interestingly, paramyosin is a heat-labile protein so the clinical relevance of paramyosin would be limited to some Asian populations such as Japanese and Koreans where mollusks and abalone are served as sashimi.

Major Echinoderm Allergens in Asian Population

Although phylogenetically distinct from crustaceans, mollusks and fin fish, echinoderms including starfish, sea cucumbers and sea urchins are also popular seafood delicacies in China, Japan and Korea. For instance, Japanese consume eggs of starfish and sea urchin roe called "*uni*." Worldwide consumption of sea urchin has been increasing, and several case reports of anaphylaxis to sea urchin roe or upon stinging by starfish were published (72, 73). A 118-kDa protein in sea urchin roe was presented as a potential allergen by immunoblotting, which was identified as major yolk protein by MALDI-TOF mass spectrometry (74).

Insect Consumption and Cross-Reactivity With Shrimp

Insects have become a unique diet in China under the cultural influence of traditional Chinese food and medicine. The consumption of oil-fried, water-boiled or ground silkworm pupa is common in China whereas silkworms have been used as therapeutics in traditional medicine and also commonly consumed after boiling with soybean sauce in Korea. Although insects are not widely consumed in western countries at present, edible insects have gained attention as a novel and sustainable food for protein sources worldwide due to the worry about food scarcity.

However, there is increasing recognition about allergy to insects. From 1980 to 2007, 61 (17%) of 358 reported cases of anaphylaxis were triggered by insects including silkworm pupas, cicada pupas, grasshoppers, locusts and hawkmoth (75). Over 1,000 patients experienced anaphylactic reactions following consumption of silkworm pupa annually in China, and foreign tourists also experienced anaphylactic shock after eating silkworm (76). Considering tropomyosin as a pan-allergen

in invertebrates including crustaceans, mollusks, dust mites and cockroaches, shrimp-allergic patients are at risk of insect allergy due to cross-reactivity between allergens. As early as 1996, Leung et al. reported co-sensitization among insects, cockroach, fruit fly and shrimp (77). More recently, Jeong et al. (78) reported among 15 patients diagnosed with silkworm pupa allergy that 11 (73.3%) exhibited sIgE reactivity to shrimp and crab by ImmunoCAP (≥0.35 kUA/L) and 53.3% of patients' sera reacted to silkworm pupa tropomyosin. Kamemura et al. (79) also demonstrated strong positive correlation between shrimp- and cricket-specific IgE responses by the IgE crosslinking-induced luciferase expression (EXiLE) assay. Similarly, Broekman et al. (80) showed that all 15 shrimp-allergic patients were also sensitized to mealworm. There was similar basophil reactivity of these shrimp-allergic patients to all tested insect species including mealworm, cricket, grasshopper and moth. By means of immunoblot and nano LC-MS/MS analyses, insect extracts were found to contain a considerable number of arthropod allergens including tropomyosin, arginine kinase, myosin light chain and triosephosphate isomerase. These allergens probably accounted for cross-reactivity between shrimp and insects. Therefore, some shrimp-allergic subjects may also be allergic to insects.

Major Fish Allergens in Asian Population

Unlike shrimp and crab where regional availability largely governs the species consumed, many fish species such as cod, salmon and tuna were distributed and consumed worldwide. In addition to these imported species, many inland Asian countries rely on freshwater aquaculture. Freshwater fishes such as carp, tilapia and catfish are stable food sources in many Asian populations. Freshwater fishes usually have a muddy taste and contain bacteria and parasites that impose food safety concern when they are eaten raw. Heat-labile fish allergens such as enolase and aldolase may thus be less relevant in allergy to freshwater fishes (**Table 2**). Our team has recently identified parvalbumin as the major allergen *Cten i* 1 in grass carp (*Ctenopharyngodon idella*) using sera from 69 subjects with IgE-mediated fish allergy (81). *Ctenopharyngodon idella* is the most commonly consumed freshwater fish species in Hong Kong, which is phylogenetically close to the common carp (*Cyprinus carpio*). By inhibition ELISA, we neatly illustrated stronger IgE inhibitory effect against *Cten i* 1 by *Cyp c* 1 (parvalbumin from common carp, ∼80% inhibition) when compared to *Gad m* 1 (parvalbumin of codfish) and *Sal s* 1 (parvalbumin of salmon). These findings could be explained by stronger allergenicity for parvalbumin of freshwater fishes than seawater fishes and/or stronger avidity of IgE to freshwater fish parvalbumin. On the other hand, Reuthers et al. (82) analyzed the IgE reactivity of 16 freshwater fishes available in Vietnam with sera from 18 Australian and three European patients with history of fish allergy. Concordantly, parvalbumin was found to be the major allergen in all freshwater fishes. Several isoforms of parvalbumin were detected in most fish species.

Apart from parvalbumin, Liu et al. (83) detected IgE binding in 10 proteins sized from 17 to 114 kDa from tilapia (*Oreochromis mossambicus*). The sensitization rate of subjects to these proteins ranged from 20 to 80%. All patients reacted a 32 kDa-protein which was identified as tropomyosin (*Ore m* 4) by LC-ES-MS/MS. *Ore m* 4 shared 58.8% sequence similarity as tropomyosin from northern shrimp, but 87.7% homology with human tropomyosin isoform 5. It should be noted that any protein resembling human proteins appear to have limited allergenic potential. In this patient group, 6 of 10 subjects suffered from inflammatory bowel disease (IBD). It is possible that anti-tropomyosin IgE antibody cross-reacted with gut proteins to result in IBD. It also raised a query if tropomyosin is a clinically relevant allergen in fish allergy.

EFFECT OF UNIQUE DIETARY HABITS IN ASIAN POPULATION TO ALLERGEN SENSITIZATION PROFILE

Raw Fish Consumption and Collagen Sensitization

Collagen was first identified as a fish allergen in a Japanese study (50), and subsequently regarded as a pan-allergen in this population. Half of 36 fish-allergic Japanese patients reacted to collagen extracted from Pacific mackerel, in comparison to only 16 (44%) patients who were positive to parvalbumin (84). In addition, 13 (36%) subjects were mono-sensitized to collagen. Collagen from Pacific mackerel also significantly inhibited IgE binding to heated crude extract of 22 fish species by 87–93%. In contrast, the sensitization rate to collagen was low in European and Australian populations (52, 85). This much higher rate of collagen sensitization together with strong IgE inhibition in Japanese might be explained by their traditional dietary practice of eating raw fish sashimi or sushi. The risk of collagen sensitization was low in cooked fish, while the insoluble intact collagen found in raw fish might be a potent sensitizing allergen due to its resistance to gastric acid digestion (84).

Fish Roe Consumption and Vitellogenin Sensitization

Fish roes are nutritious food commonly consumed in Japan. Salmon roe, also known as salmon caviar, red caviar, rainbow trout roe and ikra, is a common Japanese cuisine. Fish roe is the fifth most common food allergen source in Japan, and it is mandatory in the Japanese food sanitation law to label fish roe as a possible food allergen. In a retrospective study, half of 68 Japanese subjects reacted to salmon roe on oral food challenge (86). Analysis of sera collected from 20 patients with salmon roe allergy found that β'-component (β'-c) was the major allergen (53). Nine (45%) of 21 sera reacted to lipovitellin. Both lipovitellin and β'-c are degradation fragments of vitellogenin which issynthesized in fish liver and carried to oocytes through bloodstream. β'-c was also characterized as a major fish roe allergen in teleostean such as large yellow croaker (*Pseudosciaena crocea*) that is widely consumed in China (87). In the contrary, fish roe allergy is rare in western countries. When there is popular consumption of fish roes from sturgeon, paddlefish, cod, lumpfish, capelin, herring and salmon worldwide, there were

increasing number of cases with fish roe allergy in USA, Portugal, Spain and Finland (88–91).

Crustacean Body Parts Consumption Leading to Diversified Allergen Sensitization

Over the past decades, we found more reports on the profiling and characterization of allergens from shrimp as a prototype for shellfish. Tropomyosin has long been regarded as a major crustacean allergen. Over 80% of European shellfish-allergic subjects were sensitized to tropomyosin (92, 93). However, our recent study suggested that only slightly over half of subjects with challenge-proven shrimp allergy were sensitized to tropomyosin (94). Such low sensitization rate to tropomyosin was also reported in Thai (34.2%) and Japanese (37%) (95, 96), supporting that tropomyosin might not be the major allergen in Asians. We further demonstrated that Chinese patients were sensitized to multiple shrimp allergens, with troponin C and fatty acid-binding protein being the major allergens (97, 98).

One possible reason for such diversified sensitization profiles in different Asian populations might be due to the consumption practice for non-muscle body parts of shrimp and crab. While western populations mainly consume muscle of shelled shrimps, shrimp is always served head-on and shell-on in East and Southeast Asia when the shell, cephalothorax (containing brain, heart, stomach and bladder), ovaries and hepatopancreas are consumed together with shrimp muscle. The best example is hemocyanin that is found mainly in shrimp cephalothorax but not muscle. In addition, Khanaruksombat et al. (99) reported novel allergens from muscle, shell, hepatopancreas and ovaries of banana shrimp, *Fenneropenaeus merguiensis*, that is commonly consumed in Thailand. They detected four immunoreactive bands from shell extract and six immunoreactive bands from ovarian extracts at different vitellogenic stages. On top of arginine kinase and sarcoplasmic calcium-binding protein, these investigators identified a novel allergen called glyceraldehyde-3-phosphate dehydrogenase from the shell. Interestingly, different allergens including vitellogenin, ovarian peritrophin 1 precursor, β-actin and 14-3-3 protein were detected at different stages of ovarian development. It is noteworthy that vitellogenin is a female specific protein also found in the hemolymph of most crustacean species, and its degradation fragments lipovitellin and β'-c were defined as major fish roe allergens. This study highlighted the presence of IgE-binding proteins in both shrimp muscle and organs.

Fermented Shrimp Processing and Its Effect on Shrimp Allergenicity

Fermented foods such as kimchi and *saeujeot*, a salted and fermented shrimp product, are popular food items in Korea and some other Asian countries. *Saeujeot* is ripened primarily by proteolytic enzymes present in shrimp, and this process confers characteristic flavor and aroma in this food item. Although being heat stable, the allergenicity of tropomyosin decreased during fermentation of shrimps. Park et al. (100) demonstrated that tropomyosin could not be detected after 6 days of fermentation at 25°C, 10 days at 15°C and 30 days at 5°C. More importantly, the binding ability of tropomyosin in *saeujeot* to patient sera decreased gradually during fermentation process. This binding ability decreased further and faster at higher fermentation temperature, which might be caused by the activity of a trypsin-like enzyme that decomposes tropomyosin. Kim et al. (101) further investigated the allergenicity of *saeujeot* at various temperatures (25, 15, and 5°C) and salt concentrations (25, 15, and 10%). They showed that the binding ability of tropomyosin decreased faster at higher temperature and lower salt concentration. During fermentation, the allergenicity of *saeujeot* increased initially as tropomyosin dissolves in salt, while it is subsequently decomposed by enzymes.

FUTURE PERSPECTIVES

The clinical features of seafood allergy in Asia are distinct from the rest of the world. It is important to acknowledge such differences and to adapt our management strategies accordingly. As illustrated in peanut allergy, the key allergenic components were different in populations with high and low prevalences of peanut allergy (102). In shellfish, tropomyosin might be the key allergen in western cohorts while it is important to include other allergens such as troponin C and fatty acid-binding protein in Asians (62, 63). Fish collagen or other heat-labile proteins such as enolase and aldolase would be useful in fish allergy diagnosis in regions where raw fish consumption is common. We have also learnt that shrimp cephalothorax and fish roe can be important allergen sources, and these extracts should be standardized and made available for the clinical diagnosis of seafood allergy. In this review, we illustrated how the diagnosis of seafood allergy can be improved by studying clinically relevant or diet-related species in patients with mono-sensitization to specific seafood species. The advancement of molecular allergology prompted the identification of novel or species-specific allergens that allows us to derive more comprehensive panels for component-resolved diagnosis. Novel diagnostic platforms such as basophil activation test or EXiLE assays can also be adopted to test in-house allergen extracts in a standardized manner (94). Nonetheless, there are knowledge gaps over the prevalence and natural history of seafood allergy in Asian adults as well as the roles that dust mites and other environmental factors play in primary allergen sensitization. More challenge-based studies are also needed to identify novel seafood allergens in this region. These new scientific evidences will ultimately improve our clinical management strategies for seafood allergy.

AUTHOR CONTRIBUTIONS

CW and NL prepared the manuscript. AL, GW, and TL critically revised the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REVIEW
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Mosquitoes: Important Sources of Allergens in the Tropics

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There are more than 3,000 mosquito species. *Aedes aegypti*, *Ae. communis*, and *C. quinquefasciatus* are, among others, three of the most important mosquito allergen sources in the tropics, western, and industrialized countries. Several individuals are sensitized to mosquito allergens, but the epidemiological data indicates that the frequency of sensitization markedly differs depending on the geographical region. Additionally, the geographical localization of mosquito species has been affected by global warming and some mosquito species have invaded areas where they were not previously found, at the same time as other species have been displaced. This phenomenon has repercussions in the pathogenesis and the accuracy of the diagnosis of mosquito allergy. Allergic individuals are sensitized to mosquito allergens from two origins: saliva and body allergens. Exposure to saliva allergens occurs during mosquito bite and induces cutaneous allergic reactions. Experimental and clinical data suggest that body allergens mediate different manifestations of allergic reactions such as asthma and rhinitis. The most studied mosquito species is *Ae. aegypti,* from which four and five allergens of the saliva and body, respectively, have been reported. Many characterized allergens are homologs to arthropod-derived allergens, which cause strong cross-reactivity at the humoral and cellular level. The generalized use of whole body *Ae. communis* or *C. quinquefasciatus* extracts complicates the diagnosis of mosquito allergy because they have low concentration of saliva allergens and may result in poor diagnosis of the affected population when other species are the primary sensitizer. This review article discusses the current knowledge about mosquito allergy, allergens, cross-reactivity, and proposals of component resolved approaches based on mixtures of purified recombinant allergens to replace saliva-based or whole-body extracts, in order to perform an accurate diagnosis of allergy induced by mosquito allergen exposure.

Keywords: mosquito allergy, allergens, tropics, IgE, *Aedes aegypti*, cross reactivity

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INTRODUCTION

Mosquitoes are insects that belong to the family Culicidae, which includes more than 3,000 species distributed worldwide. Some species have the ability to adapt to different climatic conditions. Four species, *Culex pipiens*, *Culex quinquefasciatus*, *Aedes aegypti,* and the genera Anopheles have virtually populated all the planet and induce allergic reactions in atopic individuals (1).

Mosquito allergy occurs worldwide and is common in tropical and subtropical regions where mosquitoes are abundant, since the climatic conditions at these latitudes favor their life cycle and proliferation (2, 3), and increase the chances of interaction with humans. Early efforts to identify

mosquito allergens focused mainly on the saliva because it was believed that biting was the unique mechanism of exposure and sensitization. However, some evidence suggests that proteins from the insect's body may remain in the environment as aerosols or in the dust after they die and induce and allergic responses when they are inhaled by atopic individuals, similarly as house dust mites (HDMs) do.

Mosquito allergy seems to be highly prevalent and variable, although there is not enough data to support such affirmation. Diagnosis criteria is different, dependent of the study design or clinicians team. In some studies, the diagnosis of mosquito allergy was defined by bite reactions or in severe cases, anaphylaxis and systemic symptoms after a witnessed mosquito bite. Diagnosis was also made in some cases by SPT to mosquito allergen extract or positive serum to mosquito saliva IgE (4). In Monterrey City, Mexico, a cross-sectional study reported that 82% of patients admitted to the allergy service had specific IgE to mosquitoes, although only 2.5% of them showed positive skin reactions (5). In a study performed in India, 47% of the population with asthma and/or allergic rhinitis were sensitized to mosquito allergens, as determined by skin prick tests (SPT), serum specific IgE antibodies and bronchial provocation tests with whole mosquito body extracts (6). In Guangzhou, China, a study showed that in a cohort of 7,047 allergic patients, 4% of them had detectable specific IgE levels to mosquito allergens, ranging from $\geq$0.35 to $<$ 3.5 IU/ml in most of the patients, with peaks of sensitization at age between 15 and 18 years (7).

About 20 IgE binding proteins are contained in whole body extracts or the saliva from *Ae. aegypti*, but only 10 have been recognized as allergens in the databases (8, 9). Allergens from the saliva induce cutaneous reactions or a systemic response, that rarely occur (10–13). Body allergens could be contained in emanations and mosquito detritus and, when inhaled, induce variable immune responses (14, 15). A small number of mosquito allergens have been obtained and characterized. More research remains to be performed to establish the complete allergenic spectrum of *Ae. aegypti* and other species.

Studies on the cross-reactivity among different mosquito species, and with other sources of allergens, are scarce. However, an important degree of cross-reactivity between mosquitoes and other arthropods is reported (9, 16). We have found that sera obtained from a cohort of patients residing in the Caribbean island of Martinique suffering from allergic respiratory symptoms after the inhalation of HDM allergens, recognized allergens from *Ae. aegypti* (16). These findings suggest that *Ae. aegypti* contains allergens that induce a Th2 response and subsequent allergic symptoms, or could modulate the response originally established against arthropods.

High occurrence of mosquitoes at patient's homes seems to reflect a higher prevalence of sensitization and may explain a more severe cutaneous reaction during SPTs. In a study performed on a south American population sensitized to cockroaches and mosquitoes, Sanchez et al. (17) found that the size of the wheal generated during SPTs with mosquito extracts is positively correlated with the density of these insects at their homes and directly related with allergy to HDMs. This finding is similar in other tropical countries where high occurrence of mosquitoes and HDMs results in high prevalence of allergic sensitization (18). The observations open questions about the magnitude of the clinical impact produced by sensitization to mosquitoes and postulate the need for developing diagnostic tests to properly identify individuals with mosquito allergy (19). In this context, the comparison of mosquito prevalence and the frequency of sensitization to their allergens in tropical and other regions around the world should be further addressed.

MOSQUITO SPECIES: GEOGRAPHICAL DISTRIBUTION AND THEIR RELATIONSHIP WITH ALLERGIES

Mosquitoes are arthropods that belong to the class Insecta, order Diptera and members of a family of the nematocerid flies Culicidae. Two subfamilies are widely accepted within the family Culicidae: Anophelinae and Culicinae. Some authors have proposed a third subfamily, Toxorhynchitinae, which includes only one genus (1). Nearly 400 and 2,600 species are included in Anophelinae and Culicinae, respectively. The females of many species of mosquitoes require blood-feeding to reproduce, for which they bite the skin, inject saliva, and then suck blood from vessels (20). Lysozymes, antibacterial glucosidases, anticoagulants, antiplatelet aggregating factors, and vasodilators are molecules contained in mosquito saliva (21–23). Some of these substances induce allergic skin reactions (10–13). We have hypothesized that non-salivary allergens might be contained in emanations and detritus of mosquitoes, and when inhaled, induce respiratory allergic responses (9).

The mosquito species distributed worldwide easily adapt to different environmental conditions helping them to distribute in nearly any latitude (1). Distribution of mosquitoes is generalized to three main geographical locations: Cosmopolitan, Old and New world. In all of these categories, there are species associated with allergic responses. Cosmopolitan: *Anopheles (An.) stephensi, An. minimus, An. sinensis, Ochlerotatus (Oc.) triseriatus, Oc. hendersoni, Culex (Cx.) quinquefasciatus, Cx. tritaeniorhynchus, Cx. pipiens, Cx. pipiens pallens,* and *Cx. tarsalis*. Old world (Africa, Asia, and Europe): *Aedes (Ae.) aegypti, Ae. vexans, Ae. communis, Ae. togoi, Ae. albopictus,* and *Ae. triseriatus*. New world (America): *Culiseta inornate* (**Table 1**).

Although several environmental factors affect the geographical distribution of mosquitoes, the main ones are temperature, humidity, rains, and solar radiation. As a result of global warming, the distribution of some mosquito species has already changed, and they found ways to move toward other geographical areas. This behavior apply for mosquitoes and other insects as more tropical species have invaded temperate habitats, and temperate species have disappeared when their natural habitats have become warmer (24, 25). Anthropic intervention such as urbanization and transportation also plays an important role (26). For instance, *Ae. aegypti* originated in the forest areas of sub-Saharan Africa as a "wild," black-pigmented insect biting species *Ae. aegypti formosus*. Facilitated by human transportation and environmental conditions a new sub-species, *Aedes aegypti (Ae. aegypti),* evolved (27, 28) and is present in

TABLE 1 | Taxonomical classification and distribution of the main mosquito species associated with mosquito allergy.

Subfamily Return Tribe	Genera	Number of subgenera	Number of species	Distribution	Species associated with mosquito allergy
Anophelinae	*Anopheles*	7	455	Cosmopolitan	*Anopheles (An.) stephensi, An. minimus, An. Sinensis*
	Bironella	3	8	Australasian	
	Chagasia	-	4	Neotropical	
Culicinae					
Aedeomyiini	*Aedeomyia*	2	6	Afrotropical, Australasian, Oriental, Neotropical	
Aedini	*Aedes*	23	363	Old world, Nearctic	*Aedes (Ae.) aegypti, Ae. vexans, Ae. communis, Ae. togoi, Ae. albopictus, Ae. Triseriatus*
	Argimeres	2	58	Australasian, Oriental	
	Ayurakitia	-	2	Oriental	
	Borichinda	-	1	Oriental	
	Eretmapodites	-	48	Afrotropical	
	Haemagogus	2	28	Principally neotropical	
	Heizmannia	2	39	Oriental	
	Ochlerotatus	22	550	Cosmopolitan	*Ochlerotatus (Oc.) triseriatus, Oc. Hendersoni*
	Opifex	-	1	New Zealand	
	Psorophora	3	48	New world	
	Udaya	-	3	Oriental	
	Verrallina	3	95	Principally Australasian, Oriental	
	Zeugnomyia	-	4	Oriental	
Culicini	*Culex*	23	763	Cosmopolitan	*Culex (Cx.) quinquefasciatus, Cx. tritaeniorhynchus, Cx. pipiens, Cx. pipiens pallens, Cx. Tarsalis*
	Deinocerites	-	18	Principally neotropical	
	Galindomyia	-	1	Neotropical	
	Lutzia	3	7	Afrotropical, Australasian, Oriental, Neotropical, Palearctic oriental	
Culisetini	*Culiseta*	7	37	New world, Nearctic	*Culiseta inornata*
Ficalbiini	*Ficalbia*	-	8	Afrotropical, Oriental	
	Mimomyia	3	44	Afrotropical, Australasian, Oriental	
Hodgesiini	*Hodgesia*	-	11	Afrotropical, Australasian, Oriental	
Mansoniini	*Coquillettidia*	3	57	Old world, Neotropical	
	Mansonia	2	23	Old world, Neotropical	
Orthopodomyiini	*Orthopodomyia*	-	38	Afrotropical, Nearctic, Neotropical, Oriental, Palearctic	
Sabethini	*Isostomyia*	-	4	Neotropical	
	Johnbelkinia	-	3	Neotropical	
	Kimia	-	5	Oriental	
	Limatus	-	8	Neotropical	
	Malaya	-	12	Afrotropical, Australasian, Oriental	
	Maorigoeldia	-	1	New Zealand	
	Onirion	-	7	Neotropical	
	Runchomyia	2	7	Neotropical	
	Sabethes	5	38	Neotropical	
	Shannoniana	-	3	Neotropical	
	Topomyia	2	54	Principally Oriental	
	Trichoprosopon	-	13	Neotropical	
	Tripteroides	5	122	Principally Australasian, Oriental	
	Wyeomyia	15	140	Principally neotropical	
Toxorhynchitini	*Toxorhynchites*	4	88	Afrotropical, Australasian, Neotropical, Palearctic oriental, Oriental	
Uranotaeniini	*Uranotaenia*	2	265	Afrotropical, Australasian, Oriental, Neotropical	

Modified from (1).

North, Central and South America, Africa, Asia and Oceania (29). It is very abundant throughout tropical and subtropical regions of America, Africa, and Asia, as well as in the Indian Ocean islands, and northern Australia (30).

Aedes spp.

Ae. aegypti and *Ae. albopictus* are the most important species within this genus. Other Aedes species such as *Ae. vexans* (31), are tightly associated to allergic sensitization to mosquito bites. *Ae. aegypti* and *Ae. vexans* usually share their geographical distribution and are present almost worldwide. *Ae. aegypti* is arguably the most studied mosquito species as an allergenic source. Four salivary and six non-salivary allergens from this species have been deposited in the WHO/IUIS Allergen Nomenclature Sub-Committee (http://www.allergen.org). *Ae. aegypti* is rapidly expanding its geographical distribution and is highly concentrated in the tropics and subtropics (29) and have developed a preference for biting humans (32, 33), probably by an evolutionary over-expression of odorant receptors (34). Frequency of sensitization to *Ae. aegypti* varies depending on the region and the nature of the preparation used for diagnosis. Saliva-based preparations are probably more reliable to identify patients allergic to mosquito bites but might not be useful when sensitization occurs to non-salivary allergens. In a cohort of 34 allergic patients residing in the tropical island of Martinique, a prevalence of 65% of IgE reactivity to whole body *Ae. aegypti* extract was found (21). In Monterrey, Mexico, the frequency of IgE sensitization to *Ae. aegypti* was reported in 17.6% (5), similar to mosquito sensitization in a ∼18 years old allergic population from Guangzhou, China (7). *Ae. albopictus* has become a new threat to human health as it is getting spread to new tropical, sub-tropical and temperate areas (18, 35) where it is an epidemic driver of certain diseases (36). Only two allergens from *Ae. albopictus*, Aed al 2, and Aed al 3, are in the allergen database and reports of frequency of sensitization is scarce or non-existing.

Culex quinquefasciatus

Together with Aedes, species from Culex genera are above all other species as allergen sources. *C. quinquefasciatus* is a peridomestic insect that lives relatively farer from humans than *Ae. aegypti*. Native from west Africa, it feeds from birds, mammalians, and humans (37) and has spread out worldwide by commercial sailing, to warmer and temperate tropical and sub-tropical regions (38). At least 8 IgE reactive proteins have been detected in the saliva and 15 in whole body extracts from *C. quinquefasciatus* (15, 31) but only two allergens from this species, Cul q 2 and Cul q 3, have been reported in the databases (19). Epidemiologic data about allergy to *C. quinquefasciatus* is scarce. Seven out of 14 (50%) individuals from United States, Canada, Germany, Japan, and Switzerland who experienced systemic allergic reactions to mosquito bites were sensitized to this species (10). The high number of potential allergens found in whole body extracts of *C. quinquefasciatus* indicates that the role that this species may have in mosquito bite allergy or other clinical manifestations of allergy deserves to be studied.

An increase in the frequency of allergic sensitization to mosquitoes is expected to occur as a result of the environmental changes that have led to a global spreading of these insects. Temperature, relative humidity, and precipitations are the main factors that affect mosquito development, reproduction, and mortality. Temperature and relative humidity positively affect some mosquito species (39). High precipitations increase their population by maintaining their breeding (40). Allergies induced by mosquitoes and vector-borne diseases will become bigger threats for public health. The study of the pathophysiology and worsening of mosquito allergy will help to properly counteract the potential complications that will arise as a result of the increasing exposure to them.

CHARACTERIZED MOSQUITO ALLERGENS

Mosquito allergens are divided in two main groups: (a) salivary allergens (10) and (b) body-derived allergens (8). Exposure to allergens from either group results in different clinical manifestations of mosquito allergy. Salivary allergens are mainly related to cutaneous symptoms caused by mosquito bites. We hypothesized that body allergens induce respiratory allergic symptoms after inhalation of mosquito detritus (9, 16).

Saliva Allergens

Identification of salivary allergens is a difficult task and usually requires the extraction of saliva from the live mosquito or postmortem excision of the salivary gland which is used as the raw material to prepare allergenic extracts (41). Both methods are experimentally difficult (13, 41, 42) and result in low protein content. As an alternative, whole-body mosquito extracts could be used but salivary allergens are poorly represented in such preparations.

About 16 IgE-reactive bands (16-95 kDa) were detected by immunoblotting when saliva and salivary gland extracts from 10 different worldwide distributed mosquito species were analyzed (31). Sera from mosquito allergic individuals have specific IgE against 35.5, 32.5, and 22.5 kDa proteins present in the saliva of *C. quinquefasciatus* (42), and 14 proteins in salivary glands of *Aedes togoi*, *Culex tritaeniorhynchus,* and *C. pipiens pallens* with molecular weights ranging from 23 to 93 kDa (13). Some of these proteins induced an IgG1 response when used as recombinant molecules to immunize mice.

Some salivary allergens have been further characterized comprising groups 1-4 (**Table 2**). Usually, they needed to be produced as recombinant proteins because obtaining the natural version is a difficult task.

Group 1 Mosquito Allergens

The saliva apyrase (ATP di-phosphohydrolase) Aed a 1, from *Ae. aegypti,* is the only allergen from group 1 that has been accepted by the WHO/IUIS Allergen Nomenclature Sub-Committee. It corresponds to a 68 kDa enzyme with homology with the 5′-nucleotidase enzyme family (43) and interferes with platelet aggregation in human blood by hydrolyzing ADP and ATP released by the platelets and other cells (44). About 29% of Canadian individuals sensitized to mosquito bites had positive SPT to rAed a 1 (11). However, when tested in an allergic

TABLE 2 | Reported mosquito allergens.

Allergen	Biological function	Produced as recombinant	Frequency of reactivity (% positives)		Species with homolog proteins/cross-reactive allergens*
			IgE	Skin prick test	
			Salivary allergens		
Aed a 1	Apyrase	rAed a 1	—	29-43	*Aedes albopictus*: **Aed al 1** *Tabanus yao*: **Tab y 1**
Aed a 2	Salivary D7 protein	rAed a 2	43	11	*Aedes albopictus*: **Aed al 2** *Culex quinquefasciatus*: **Cul q 2** *Anopheles darlingi*: **Ano d 2**
Aed a 3	Undefined 30 kDa salivary protein	rAed a 3	—	32	*Aedes albopictus*: **Aed al 3** *Culex quinquefasciatus*: **Cul q 3**
Aed a 4	α-glucosidase	rAed a 4	36	—	*Culex quinquefasciatus Aedes albopictus*
			Body derived allergens		
Aed a 5	Sarcoplasmic Ca+ (EF-hand) binding protein	No	26.2	—	*Aedes albopictus Culex quinquefasciatus Anopheles stephensi Anopheles albimanus Anopheles sinensis*
Aed a 6	Porin 3	No	33.3	—	*Culex quinquefasciatus*
Aed a 7	Undefined protein	No	26.6	—	—
Aed a 8	Heat Shock cognate protein-70	rAed a 8	60	—	*Alternaria alternata*: **Alt a 3** *Aspergillus fumigatus*: **Asp f 12** *Dermatophagoides farinae:* **Der f 18** *Dermatophagoides pteronyssinus:* **Der p 28** *Malassezia sympodialis:* **Mala s 10** *Penicillium citrinum:* **Pen c 19** *Corylus avellana:* **Cor a 10** *Blomia tropicalis Vespa affinis* etc.
Aed a 10	Tropomyosin	rAed a 10.0101 rAed a 10.0201	33.3	—	*Anisakis simplex:* **Ani s 3** *Blattella germanica:* **Bla g 7** *Dermatophagoides farinae:* **Der f 10** *Dermatophagoides pteronyssinus:* **Der p 10** *Blomia tropicalis:* **Blo t 10** *Chironomus kiiensis*: **Chi k 10** *Crangon crangon*: **Cra a 1** *Exopalaemon modestus:* **Exo m 1** *Haliotis laevigata:* **Hal l 1** *Helix aspersa:* **Hel as 1** *Homarus americanus:* **Hom a 1** *Litopenaeus vannamei*: **Lit v 1** *Penaeus monodon:* **Pen m 1** *Periplaneta americana:* **Per a 7** *etc.*
Aed a 11	Lysosomal aspartic protease	No	40	—	**Aspartic proteases in [#]:** *Aspergillus fumigatus:* **Asp f 10** *Blattella germanica:* **Bla g 2** *Periplaneta americana*: **Per a 2** *Solanum tuberosum:* **Sola t 2**

**Allergen names are shown in bold and included only when reported in the WHO/IUIS Allergen Nomenclature Sub-Committee.*
[#]Allergens reports as Aspartic proteases, not "Lysosomal aspartic protease" as in Ae. Aegypti.

population from the tropics, living in urban and sub-urban areas, the IgE frequency of reactivity increased to 60% (19). B cell epitopes seem to be contained in the 150-562 amino acid region and react with the IgE and IgG from allergic individuals (45). Homolog molecules or apyrase enzymatic activity have been detected in the saliva from *Ochlerotatus triseriatus, Ochlerotatus hendersoni* (46), and *Ae. albopictus* (31, 47).

Group 2 Mosquito Allergens

It corresponds to allergens that belong to the family of proteins called D7, which are required by mosquitoes for feeding and reproduction, and are released together with the saliva during biting. They have structural homology with the protein THP12 from *Tenebrio molitor*, which is part of the family of pheromone-binding proteins and odorants and help transporting hydrophobic molecules (48). Allergens within this group have been reported in the WHO/IUIS allergen database from *Ae. aegypti* (49) and in *Ae. albopictus*, *An. dirus,* and *C. quinquefasciatus* (19). This group could also be present in other Aedes species and *O. triseriatus* (31).

Aed a 2, from *Ae. aegypti,* is a multi-domain protein with a N-terminal and a C-terminal domain that binds leukotrienes

and biogenic amines released as a mechanism of protection in individuals that are getting bitten (50). In a group of 15 mosquito bite allergic individuals residing in the tropics the frequency of reactivity was 100%, studied by immunoblotting using salivary gland extracts (19). However, in a North American population seems to be 11% (31). Recombinant Aed a 2 expressed in insect cells infected with baculovirus retains the IgE-binding capacity and allergenicity, and immunogenicity as seen in immunized mice (51), suggesting that it can be used as a replacement of the natural protein.

Group 3 and 4 Mosquito Allergens

The WHO/IUIS allergen database reports allergens in groups 3 and 4 from the mosquito species *Ae. aegypti* (52, 53), *Ae. albopictus* and *C. quinquefasciatus* (19). Aed a 3 and Aed al 3 in *Ae. aegypti* and *Ae. albopictus*, respectively, are 30 kDa molecules. In *C. quinquefasciatus*, Cul q 3 is a 35 kDa molecule. Aed a 3 from *Ae. aegypti* shows collagen binding capacity and prevents its interaction with platelet glycoprotein IV, integrin α2β1 and von Willebrand factor (52). When used together with Aed a 1 and Aed a 2, about 60% of an allergic population could be accurately diagnosed (53). 40% of individuals from a tropical region react against Aed a 3. Aed a 4 is a 67 kDa α-glucosidase. About 36-46% of mosquito allergic individuals react against this allergen (19, 54).

Body-Derived Allergens

Allergic individuals have IgE against non-salivary body-derived mosquito proteins. For instance, in the subtropical city of Yazd, Iran, 33% of individuals with allergic rhinitis had positive skin test to whole body mosquito extracts (55). Similar observations were reported in India where 47% of the population with asthma and/or rhinitis were sensitized to mosquito allergens (6) and in Martinique with 65% of sensitization (16). Such observations strongly suggest that exposure to mosquito allergens occurs through the skin when the mosquito is biting, but also through the airways, leading to different manifestations of the allergic response such as asthma and rhinitis.

An important question to address is whether body-derived mosquito allergens are found in the dust or mattresses from the allergic individuals' residing places and in quantities enough to induce allergic symptoms. Although we don't know the answer yet, several studies have made important advances in this matter. To begin, extracts prepared from airborne particles collected in the homes of mosquito allergic individuals block the specific IgE reactivity of sera from such individuals to whole-body *C. quinquefasciatus* extract (14), which allows to hypothesize that mosquito allergens are present in house dust and retains antibody binding capacity. A weakness of this hypothesis is that it is based on immunoassays, and it cannot exclude that arthropod-derived allergens might be the molecules responsible of inhibiting the IgE binding capacity. It is already demonstrated that they are present in the dust from places where allergic individuals reside (56, 57). The DNA-based study of arthropod diversity in homes via high-throughput marker gene sequencing of 700 home's dust revealed that mosquito (*Aedes spp*) together with carpet beetle, dust mite and Aphid (*Aphis spp*) are common in home's dust (58). Quantitative analyses are necessary to establish whether the amounts of mosquito allergens in such samples are high enough to represent a potential primary sensitizer and inducer of allergic symptoms.

Different allergen composition has been observed depending on the sample and techniques used to detect IgE binding molecules. There are at least 11 IgE-binding proteins in whole-body *Ae. aegypti* extract, as detected by immunoblotting (16). Five of those proteins cross-react with allergens from HDM, cockroach and shrimp. Whole-body extracts are prepared by extraction with PBS and non-PBS soluble allergens could be missing. The analysis of the *Ae. aegypti* allergenome using proteomic tools revealed a set of 25 IgE-binding molecules corresponding to 10 different proteins and some of their variants or isoforms (8). Four of them were deposited in the WHO/IUIS Allergen Nomenclature Sub-Committee as Aed a 5.0101 (sarcoplasmic Ca^{+} (EF-hand) binding protein), Aed a 6.0101 (Porin 3), Aed a 7.0101 (undefined protein), Aed a 8.0101 (HSC-70), and Aed a 11.0101 (lysosomal aspartic protease). Notice that tropomyosin Aed a 10 was also identified. Only the HSC-70, Aed a 8 and tropomyosin Aed a 10 have been further studied (**Table 2**).

Group 8 Mosquito Allergens

Aed a 8 is the representative allergen of this group. Heat shock cognate protein-70 belongs to the highly conserved Heat shock protein-70 family (59), chaperones that help in protein folding maintaining their correct biological function under stress conditions (60). Homolog allergens are present in *Dermatophagoides farinae* (61) and cockroach (62). Aed a 8 reacted with the IgE in 9 out of 15 allergic individuals (60%) (8). Similar frequency of reactivity is reported for Der f 8 from *D. farinae* (61).

We obtained recombinant Aed a 8 as a 74 kDa by expression in *Escherichia coli*. Recombinant Aed a 8 inhibited 43% of the IgE reactivity of a mixture of human serum samples to the whole body extract of *Ae. aegypti*, indicating that the wild type Aed a 8 is present in such extract, and retains immunogenicity and the capacity to activate basophils. Six out of 14 sera from allergic individuals reacted to the recombinant and, when used to immunize mice, it induced specific antibody that also reacted against the natural counterpart, indicating that it retained biological activity (63).

Obtaining mosquito allergens is a difficult task, especially for proteins that are expressed in low levels, such as HSC-70 molecules. Using purified and biologically active recombinant allergens will help to overcome this problem and we strongly suggest using rAed a 8 for further analysis of mosquito allergy and study the clinical relevance of group 8 allergens in the physiopathology of mosquito allergy.

Group 10 Mosquito Allergens

Tropomyosin is a well-described allergen from diverse sources. Some of the allergenic sources are shrimps, lobsters, prawns, crabs, fish, mollusks, and snails. This allergen is also common in HDMs, helminths, cockroaches, and insects, and partially explains the existence of the cross-reactivity between them (64,

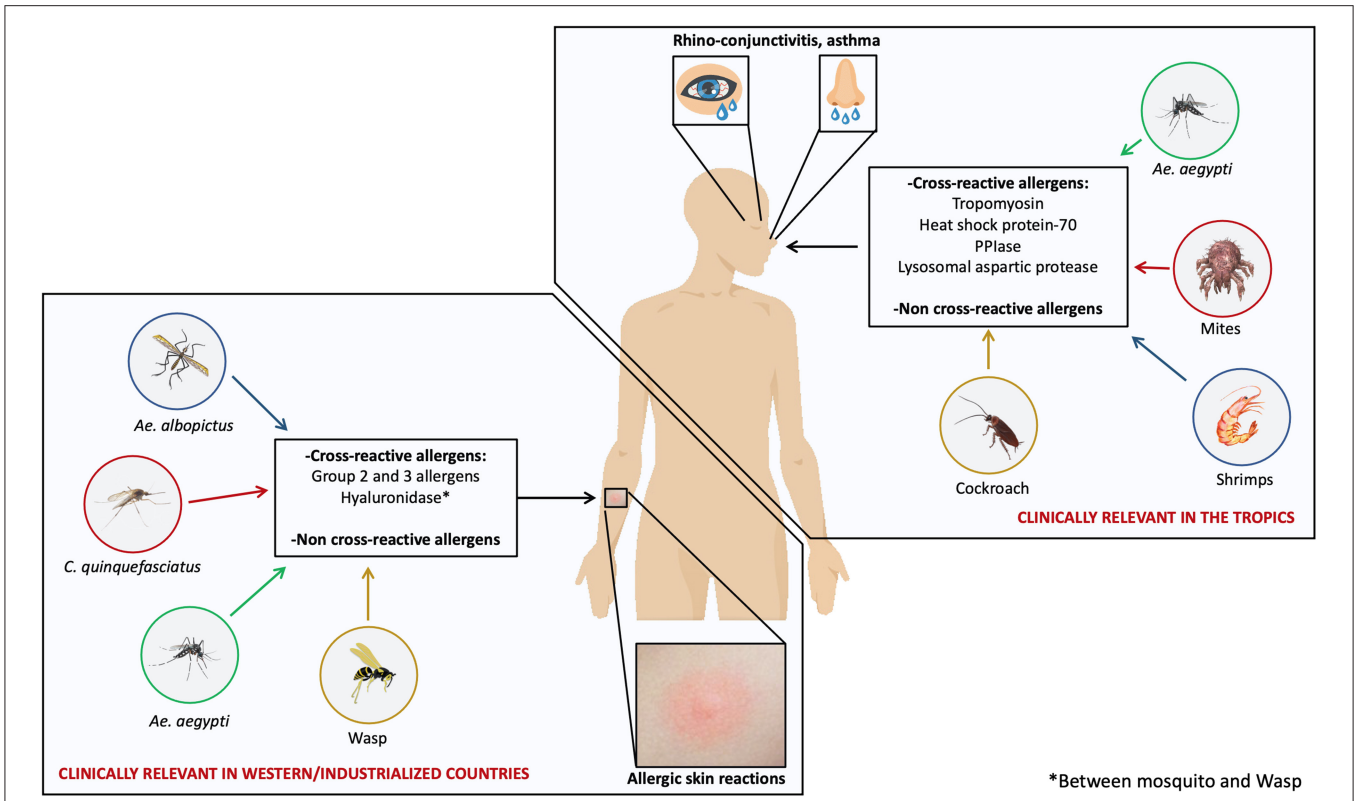

FIGURE 1 | Unique and cross-reactive mosquito allergens induce different manifestations of mosquito allergy. Mosquito species such as *Ae. aegypti*, *Ae. albopictus*, and *C. quinquefasciatus* are distributed worldwide; however, some studies suggest that two kinds of allergic reactions induced by mosquito allergens are clinically relevant depending on the geospatial location: allergic skin reactions induced by salivary allergens and respiratory reactions induced by body-derived allergens. Skin reactions are common in western and industrialized countries and respiratory reactions are relevant in tropical areas. Cross-reactivity between mosquito species and with several species within Arthropods may play an important clinical role.

65). *Ae. aegypti* has 11 genes that encode different variants, or isoforms of tropomyosin. Four of them were detected, characterized and purified (66). Two tropomyosin isoforms, Aed a 10.0101 and Aed a 10.0201 are the most abundant and 33% of a population sensitized to *Ae. aegypti* had IgE against a mixture of them (66), suggesting that they are relevant molecules involved in IgE sensitization against *Ae. aegypti* tropomyosins.

The IgE frequency of sensitization to tropomyosin is variable, but usually low. Tropomyosin from shrimp species *Penaus aztecus*, Pen a 1, binds up to 75% of shrimp-specific IgE antibodies (67, 68). In Africa and South America, the prevalence of sensitization to mite tropomyosin is ∼50% (69, 70), higher than that in developed countries (71, 72). The relatively high frequency of sensitization to tropomyosin in African and South American areas indicates that cross-reactivity with mosquito tropomyosin must be considered.

IgE CROSS-REACTIVITY MEDIATED BY MOSQUITO ALLERGENS

The apparent geospatial differences of immune and allergic response to mosquito allergens have implications in the cross-reactivity phenomena. In regions where cutaneous allergic reactions to mosquito bites is frequent, saliva-derived allergens are the main cross-reactive molecules (15, 31, 73, 74). In contrast, in tropical areas, body allergens seem to be the main proteins associated to cross-reactivity with arthropods (8, 16) (**Figure 1**). These differences have clinical implications since preparations for diagnostic and immunotherapy based on salivary allergens would make sense to consider in western and industrialized countries. The case is different for tropical and subtropical countries where species specific and cross-reactive body-derived allergens might be the best targets to focus on. It is also possible that in these regions, body-based preparations could be a more effective tool to cope with allergies caused by mosquitoes and other arthropods.

Cross-Reactivity Mediated by Saliva Allergens

Studies on animals indicate that sensitization to a mosquito salivary allergen induce antibodies that react against allergens from different mosquito species. Sera from rabbits immunized with rAed a 1 cross-react with extracts from *Ae. vexans* and *Ae. albopictus* (31). The finding of homologs of the apyrase Aed a 1 allergen in *Ae. aegypti*, *O. triseriatus,* and *O. hendersoni* indicates that this protein is conserved among several mosquito species and explains the above-mentioned observations. Similarly, immunization with rAed a 2 induces anti-sera that react with extracts of *C. quinquefasciatus*, *O. triseriatus* (46) and several

species of *Aedes* (12, 31). It is plausible to assume that saliva proteins other than group 1 and 2 allergens are involved in the cross-reactivity among mosquito species.

Several studies show a similar phenomenon in humans. Individuals from Shanghai, China, have IgE-reactivity to *Ae. vexans* allergens, although this species is not indigenous in such area (31, 73). Contrarily, *Ae. vexans* is a major pest in Winnipeg, Manitoba (Canada) where individuals allergic to mosquitoes co-react with allergens from other mosquito species not found in Manitoba (73). The sera from individuals allergic to mosquito bites in Thailand react with several broad range molecular weight proteins present in the extracts from the *C. quinquefasciatus*, *Ae. aegypti*, *Ae. albopictus,* and *An. minimus,* common mosquitoes (15).

Saliva derived allergens from mosquitoes can also cross-react with proteins from wasps. The so-called "wasp/ mosquito syndrome," involves an IgE cross-reactive 44-kDa hyaluronidase which is present in both insects (74). Cross-reactivity between salivary allergens occurs in western/industrialized countries as well as in tropical regions. However, it is necessary to evaluate the clinical implications that this may have. In countries like Canada where cross-reactivity among *Ae. vexans* and several other mosquito species is common (31, 73) and mosquito bite allergies are frequent, it is important to determine whether such cross-reactivity has implications in the physiopathology of allergic responses. However, in other regions like Brazil, cross-reactivity between endemic mosquito species also occur (48), but it involves antibodies from allergic and non-allergic individuals. This suggests that in such regions, broad sensitization to mosquito occurs but does not mean that it leads to a clinical manifestation of allergy and cross-reactivity might not be important.

Cross-Reactivity Mediated by Body-Derived Allergens

There are homolog proteins distributed in several species from the filum Arthropoda, including mosquitoes, that induce allergic reactions. It is widely accepted that in the tropics HDMs, cockroaches and shrimp are some of the most common sources of allergens (75).

in vitro studies and SPTs showing that individuals sensitized to one or several arthropod species had concomitant immunoreactivity against mosquito proteins or extracts led to the hypothesis that cross-reactivity involving allergens from mosquitoes and other sources occurs (76, 77) (**Figure 1**).

In our mentioned study with allergic individuals from Martinique (16), we identified four novel cross-reactive allergens in *Ae. aegypti* allergen extract and concluded that, these molecules could influence the manifestation of allergy to environmental allergens in the tropics. ELISA experiments showed that in this population *D. pteronyssinus*, *Litopenaeus vannamei*, *Blomia tropicalis*, and *Periplaneta americana* extracts inhibited the IgE reactivity to *Ae. aegypti* extract in 75.4-96.6%, and that the main allergen involved was tropomyosin (16), a well-known cross-reactive molecule within arthropods. Besides tropomyosin, other components are involved, especially a 17.9 kDa PPIase that has 81.1% identity in the amino acid sequence with Der f 29 allergen from *D. farinae*.

Tropomyosin is the main cross-reactivity allergen in *Ae. aegypti,* which is expressed as several variants and isoforms. Two of the more abundant are Aed a 10.0101 and Aed a 10.0201, which cross-react with rDer p 10 from *D. pteronyssinus* (78). In the Caribbean, 33% of a group of sera from allergic individuals had specific IgE to these two tropomyosins (9); a number that is evidently higher than the frequency of sensitization to tropomyosins from other sources typically observed in developed countries.

We demonstrated that cross-reactivity of *Ae. aegypti* tropomyosins leads to effector cell activation. We used basophils in the PBMCs from non-allergic donors where the membrane bound IgE was stripped away and re-sensitization with sera from allergic patients sensitized to the tropomyosin Der p 10. Challenging such cells with rDer p 10 or recombinant *Ae. aegypti* tropomyosins, induced dose dependent activation. In addition, splenocytes from mosquito tropomyosin immunized mice proliferate upon stimulus with rDer p 10 (78).

DIAGNOSIS

Whole body extracts prepared from *Ae. communis*, *C. pipiens* or *C. quinquefasciatus* are currently the main preparations used for the purpose of mosquito allergy diagnose, although their use has some disadvantages. To begin, the accuracy of the diagnosis is compromised when the primary sensitizer is a species different to the one used to prepare the allergenic extract. Different geographical regions have different local mosquito species and having the appropriate mosquito extract that works for a specific population, is mandatory to achieve an appropriate diagnosis (19), but sometimes is not possible. For instance, *Ae. communis* is endemic in northern temperate zones but poorly present in tropical countries where *Ae. aegypti* and *C. quinquefasciatus* are abundant (18). The use of *Ae. communis* extract results in poor diagnosis of mosquito allergic individuals from the tropics (19, 79). In contrast to the case in Cuba, where mosquito allergy is frequently related to *C. quinquefasciatus* bites and using a high dose of standardize extract of this mosquito species in SPTs resulted in positive results that correlated in 100% of the patients (80). Second, whole body extracts may have poor representation of saliva allergens (15, 81), which could jeopardize the accuracy of such preparations to detect allergic individuals who are sensitized to the saliva (79). Wang et al. found that the diagnosis by the detection of specific IgE using salivary extracts provide higher specificity and sensitivity than using whole body extracts (82). Alternatively, using saliva-based preparations or salivary gland extracts, may provide 80% positivity result (4). However, this is not cost effective and requires complicated procedures that result in low recovery of allergens. Using whole-body extracts appear more attractive when the affected population is sensitized to non-salivary allergens.

Using recombinant allergens is especially convenient to circumvent the above mentioned problems as they are obtained in high amounts and purity. Additionally, they have the intrinsic advantages when used as a replacement of natural extracts, as they can be easily standardized, subjected to proper quality

control analysis and allows component-resolved immunotherapy since it help to identify the set of allergens to which each individual is sensitized (83–85). Only a few recombinant mosquito allergens have been obtained and analyzed. Aed a 1, Aed a 2, and Aed a 3 have been well-characterized, obtained as recombinants and are an interesting tool to replace *Ae. aegypti* saliva since a mixture of the three allergens allows identifying 60% of the *Ae. aegypti* population allergic to mosquito bites (53). Evidently, clinically relevant mosquito allergens must be chosen to allow a better identification of allergic individuals (86). Obtaining recombinant saliva allergens from other species is also necessary to allow future development of more accurate diagnostic tests.

The situation is similar for individuals sensitized to non-salivary allergens. Very few body allergens have been detected and only two recombinant allergens from *Ae. aegypti,* rAed a 8, and rAed a 10 (9), have been produced and tested. We made some advances and proposed an alternative to replace whole body *Ae. aegypti* extracts for a mixture of three allergens, Aed a 6, Aed a 8, and Aed a 10, which may be enough to identify more than 80% of the allergic individuals (8). More efforts must be done to broadly identify and characterize saliva and body mosquito allergens from different species, obtain relevant allergens as recombinant proteins and confirm their potential as diagnostic tools in clinical studies with well-characterized populations.

CONCLUDING REMARKS

The concept of mosquito allergy should be re-evaluated as more allergens have been identified, revealing that they belong to the saliva and the insect's body. Mosquito body allergens seem to induce different types of allergic responses, such as asthma, allergic rhinitis, and probably conjunctivitis. The mechanisms of exposure to these allergens are not established yet but, may occur by inhalation of mosquito detritus suspended in the air. These observations have several implications and open many questionings: (1) Is there a relationship between the exposure to mosquito allergens and the onset of respiratory allergic reactions? (2) Do mosquito allergens induce manifestation of allergic responses different to the cutaneous or airway related symptoms? (3) Could mosquito allergens contained in the environment induce immunological responses?

The current knowledge has many unresolved issues. Only a few allergens have been identified and characterized, and they belong to a few species, mainly *Ae. aegypti* and *C. quinquefasciatus*. The diversity of mosquito species is quiet variable depending on the geographical region and it has continuously changed with global warming. Additionally, an important degree of cross-reactivity occurs among mosquitoes and several arthropod species. The effects that this phenomenon has on the pathophysiology of allergy diseases is still unknown.

The quest for answers to these questions will help to propose a more accurate definition of mosquito allergy and may pave the way to find solutions to the scientific and clinical challenges that will subsequently arise. More efforts must be done to identify and characterize saliva and mosquito body allergens from different species, obtain relevant allergens as recombinant proteins and confirm their potential as diagnostic tools in clinical studies with well-characterized populations.

AUTHOR CONTRIBUTIONS

LP conceived the idea. LP and JC contributed equally to the preparation of the draft and final manuscript. All authors contributed to the article and approved the submitted version.

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Patterns of Allergic Sensitization and Factors Associated With Emergence of Sensitization in the Rural Tropics Early in the Life Course: Findings of an Ecuadorian Birth Cohort

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Introduction: There are limited data on emergence of allergic sensitization (or atopy) during childhood in tropical regions.

Methods: We followed a birth cohort of 2,404 newborns to 8 years in tropical Ecuador and collected: risk factor data by maternal questionnaires periodically from birth; atopy was measured by skin prick test reactivity (SPT) to aeroallergens in parents, and aeroallergens and food allergens in children at 2, 3, 5, and 8 years; and stool samples for soil-transmitted helminths (STH) from children periodically to 8 years and from parents and household members at the time of recruitment of cohort children. Data on risk factors were measured either at birth or repeatedly (time-varying) from birth to 8 years. Longitudinal repeated-measures analyses were done using generalized estimating equations to estimate the age-dependent risk of positive SPT (SPT+) to any allergen or mite during early childhood.

Results: SPT+ to any allergen was present in 29.0% of fathers and 24.8% of mothers, and in cohort children increased with age, initially to mite but later to cockroach, reaching 14.8% to any allergen (10.7% mite and 5.3% cockroach) at 8 years. Maternal SPT+, particularly presence of polysensitization (OR 2.04, 95% CI 1.49–2.77) significantly increased the risk of SPT+ during childhood, while household overcrowding at birth decreased the risk (OR 0.84, 95% CI 0.72–0.98). For mite sensitization, maternal polysensitization increased (OR 2.14, 95% CI 1.40–3.27) but rural residence (OR 0.69, 95% CI 0.50–0.94) and birth order (3rd−4th vs. 1st−2nd: OR 0.71, 95% CI 0.52–0.98) decreased the risk. Time-varying exposures to agricultural activities (OR 0.77, 95% CI 0.60–0.98) and STH parasites (OR 0.70, 95% CI 0.64–0.91) during childhood decreased while anthelmintics increased the childhood risk (OR 1.47, 95% CI 1.05–2.05) of mite sensitization.

Conclusion: Our data show the emergence of allergic sensitization, primarily to mite and cockroach allergens, during childhood in tropical Ecuador. A role for both antenatal and post-natal factors acting as potential determinants of SPT+ emergence was observed.

Keywords: allergic sensitization, atopy, cohort, childhood, tropics, risk factors

INTRODUCTION

Allergen sensitization or atopy represents a predisposition of humans to generate IgE responses to biochemically heterogeneous molecules present in the environment. These molecules or allergens are derived from sources including arthropods, pollens, molds, and foods. Sensitization rates vary between populations according to host genetic factors, the allergens to which a population is exposed, and the presence of environmental exposures that modify the expression of atopy (1). Allergen sensitization can be measured in epidemiological studies either by the presence of IgE-mediated inflammation to allergen extracts *in vivo* by skin prick testing or by detection of specific IgE in blood samples. Atopy is an important risk factor for the development of common inflammatory diseases such as asthma, eczema, rhinitis, and food allergy (1, 2).

There are numerous epidemiological studies from both high-income and low and middle-income countries on rates of allergen sensitization in general population samples and in children and adults with and without evidence of allergic diseases (2–4). These studies show that the prevalence and specificities of allergen sensitization varies considerably across populations (5, 6). Although studies from tropical settings are less common, most have shown in both population-based and patient-based studies that the primary allergen sensitizers are arthropod species including dust mites and cockroaches that thrive in these warm and humid environments (6). There are few published longitudinal studies of the emergence of allergic sensitization early in the life course from tropical regions (7, 8) that have studied the role of antenatal and post-natal host and environmental factors as risk factors for allergic sensitization.

We have shown previously in a birth cohort, from a tropical rural district of coastal Ecuador, followed to 8 years of age, that childhood and or maternal STH parasites protected against the development of mite sensitization at 3 years (9), to perennial allergens at 5 years of age (10), and to any allergen when measured at 8 years of age (11). In the present analysis, we describe the development and patterns of allergic sensitization measured by allergen SPT+ to school age and explore the potential role of a range of relevant ante-natal and post-natal individual and environmental risk factors, with a particular focus on the role of exposures associated with poor hygiene, in determining the emergence early in the life course of SPT+ in this tropical setting.

Abbreviations: AIC, Akaike's information criterion; CI, confidence interval; GEE, generalized estimation equations; LMIC, low and middle-income country; OR, odds ratio; SPT, allergen skin prick testing; SPT+, allergen skin prick test positivity; STH, soil-transmitted helminths; Th2, T helper cell type 2.

MATERIALS AND METHODS

Study Area and Population

Detailed methodology of the study objectives, design, follow-up, and sample and data collection for the ECUAVIDA birth cohort study are provided elsewhere (12). Briefly, newborns whose families lived in the rural district of Quininde, Esmeraldas Province, were recruited around the time of birth at the Hospital Padre Alberto Buffoni (HPAB) in the town of Quininde between November 2005 and December 2009. The District is largely agricultural where the main economic activities relate to the cultivation of African palm oil and cocoa. The climate is humid tropical with temperatures generally ranging 23–32°C with yearly rainfall of around 2,000–3,000 mm. Inclusion criteria were being a healthy baby, collection of a maternal stool sample, and planned family residence in the district for at least 3 years.

Sample and Data Collection

Children were followed-up from birth to 8 years of age with data and samples including stools collected at baseline during the initial home visit within 2 weeks of birth and at 7 and 13 months, 2, 3, 5, and 8 years of age. Stool samples were collected from mothers during the third trimester of pregnancy and from household members during the initial home visit around the time of birth of the child. Follow-ups were done either by scheduled visits to a dedicated clinic at HPAB or by home visits. At the initial home visit, a questionnaire was administered to the child's mother by a trained member of the study team to collect data on risk factors, potential confounders, and allergic diseases. Maternal questionnaires were repeated at the time points listed above. Presence of allergic disease symptoms at 8 years of age were defined as described (11, 13).

Stool Examinations

Stool samples were examined using four microscopic techniques to detect soil-transmitted helminth eggs and larvae including direct saline wet mounts, formol-ether concentration, modified Kato-Katz, and carbon coproculture (14). All stool samples were examined using all 4 microscopic methods where stool quantity was adequate. A positive sample was defined by the presence of at least one egg or larva from any of the above detection methods. Parasite burdens with *A. lumbricoides* and *T. trichiura* were quantified as eggs per gram (epg) of stool using the results of the modified Kato-Katz method and categorized into light, moderate, and heavy intensities using WHO criteria (14) as follows: *A. lumbricoides* (light−1–4,999; moderate−5,000–49,999; and heavy—≥50,000 epg) and *T. trichiura* (light−1–999; moderate−1000–9,999; and heavy—≥10,000 epg).

Allergen Skin Prick Test Reactivity

Allergic sensitization was measured by SPTs and done on fathers and mothers of cohort children and on cohort children at 2, 3, 5, and 8 years. SPT on parents used the following allergen extracts: house dust mites (*Dermatophagoides pteronyssinus/Dermatophagoides farinae* mix) (Greer laboratories, Lenoir, North Carolina, USA), American cockroach (*Periplaneta americana*) (Greer), cat (Greer), dog (Greer), grass pollen (9 southern grass mix containing pollen from Bermuda, Kentucky Blue/June, Johnson, Meadow Fescue, Orchard, Perennial Ryegrass, Redtop, Sweet Vernal, and Timothy grasses) (Greer), fungi [New stock fungi mix containing *Acremonium strictum, Alternaria alternata, Aspergillus niger, Aureobasidium pullulans, Bipolaris sorokiniana, Botrytis cinerea, Candida albicans, Chaetomium globosum, Cladosporium sphaerospermum, Epicoccum nigrum, Fusarium moniliforme, Mucor plumbeus, Penicillium chrysogenum (notatum), Phoma betae, Rhizopus stolonifer,* and *Trichophyton mentagrophytes*] (Greer), *Alternaria tenuis* (Greer), *Blomia tropicalis* (Leti Pharma, Barcelona, Spain), and *Chortoglyphus arcuatus* (Leti) with positive histamine (10 mg/mL) (ALK-Abello, Horsholm, Denmark) and negative saline controls (ALK-Abello). SPT on children used the following 9 allergen extracts (all Greer): house dust mites (*Dermatophagoides pteronyssinus/Dermatophagoides farinae* mix), American cockroach (*Periplaneta americana*), cat, dog, grass pollen (9 southern grass mix), fungi (New stock fungi mix), egg, milk, and peanut, with positive histamine (10 mg/mL) and negative saline controls (ALK-Abello, Horsholm, Denmark). A positive reaction was defined as a mean wheal diameter (mean of longest and orthogonal diameters) at least 3 mm greater than the saline control 15 min after pricking the allergen onto the forearm with lancets (ALK-Abello). Atopy was defined as a positive reaction to any of the allergens tested. Polysensitization was defined as $\geq$2 positive tests to the same panel of 6 allergens which were tested in both children and parents.

Anthelmintic Treatments

Individuals with positive stools for STH infections were treated with a single dose of 400 mg albendazole if aged 2 years or greater and with pyrantel pamoate (11 mg/kg) if aged <2 years, according to Ecuadorian Ministry of Public Health recommendations (13). Pregnant women were offered treatment after the delivery of their child. All treatments were provided free by members of the study team.

Statistical Analysis

The original cohort was designed to study associations between STH infections and allergic outcomes with follow-up to 5 years which was later extended to 8 years (15). SPT+ to any allergen and to mite defined two longitudinal binary outcomes. SPT testing was done at 24, 36, 60, and 96 months of age with some time variation but for purposes of analyses, follow-up dates were considered fixed. We use generalized estimation equations (GEEs) to fit population-averaged models (16, 17) for effects of age, child, parental, household, and poor hygiene characteristics. The assumption of the correlation structure was that of unstructured (the most general) (16–18). Adjusted associations of longitudinal outcomes with risk factors were assessed using adjusted odds ratios and their 95% confidence intervals with significance level set to 0.05. ORs derived from these longitudinal models estimated associations between potential explanatory variables and the age-dependent risk of SPT+. Conceptually, the interpretation is equivalent to a cross-sectional OR. Longitudinal ORs allow stratification of the age-dependent risk of infection by categories of predictors—these are non-linear relationships and an OR >1 represents a higher risk of SPT+ associated with that category compared to the baseline category across all ages, assuming no interaction with age. Minimally adjusted models (for age and age^2) assessed associations of each factor with risk of SPT+. Multivariable models were built using variables with $P < 0.1$ in minimally adjusted models. Among highly correlated variables considered for inclusion in multivariable analyses (**Table 1**), the variable/s with the smallest associated quasi-likelihood under the independence model criterion (QIC) criterion for GEEs (19, 20) on the same data sample were chosen. The QIC criterion is an adaptation of the Akaike's information criterion (AIC) criterion for GEEs for model choice (18, 19). The final most parsimonious model derived on a complete data sample was subsequently fit back to the original data on as many observations as possible.

Longitudinal cohorts are subject to attrition at follow-up. We investigated patterns in missing data for SPT+ to any allergen and carried out sensitivity analyses by generating new outcomes where 1 indicated a missing observation and 0 otherwise. Sensitivity analyses to missing data are available upon request. The GEE estimation is based on missing completely at random assumption (21). However, random effects based on maximum likelihood estimation were also fit and did not produce very different estimates in terms of magnitude or precision of ORs. This type of estimation, more sensitive to distributional assumptions, was made under missing at random assumption (20). Urban-rural residence was defined by geographic boundaries. All statistical analyses were done using Stata 16 (Statacorp, College Station, Tex).

Ethical Considerations

Study protocols were approved by ethics committees in Ecuador (Hospital Pedro Vicente Maldonado, Universidad San Francisco de Quito, and Universidad Internacional del Ecuador) and UK (London School of Hygiene and Tropical Medicine). The study is registered as an observational study (ISRCTN41239086). Informed written consent was obtained from the child's mother and minor assent was obtained from the child at 8 years.

RESULTS

Cohort Participants and Characteristics

Analyses during the first 8 years of life used data from all 2,404 children with data for SPT at 1 or more of the 4 observation times from 2 years: 97.0% of children had SPT for at least one observation time and 72.7% had SPT from all 4 observation times. A total of 1,952 (81.2%) had data on SPT at 8 years. **Figure 1** shows numbers sampled for SPT between 2 and 8 years. Characteristics of the 2,404 newborns at the time of birth

TABLE 1 | Minimally age-adjusted associations between risk of allergen skin prick test positivity (SPT+) to any and to mite allergens during first 8 years of life and child, parental, household, and hygiene factors.

Variable		SPT+ to any allergen				SPT+ to mite			
	N (%)	OR	95%CI		*p*-value	OR	95%CI		*p*-value
CHILD FACTORS									
Gender									
Male	1,193 (51.2%)	1				1			
Female	1,139 (48.8%)	0.88	0.73	−1.06	0.185	0.91	0.72	−1.17	0.466
Breastfeeding									
0–6 months	232 (10.5%)	1				1			
7–12 months	956 (43.3%)	0.93	0.67	−1.27	0.628	**0.67**	**0.45**	**−0.99**	**0.044**
>12 months	1,022 (46.2%)	0.91	0.66	−1.24	0.541	0.74	0.51	−1.10	0.126
MATERNAL FACTORS									
Age (years)									
≤20	622 (26.7%)	1				1			
21–29	1,129 (48.4%)	1.14	0.91	−1.43	0.264	1.23	0.91	−1.67	0.183
≥30	581 (24.9%)	1.11	0.86	−1.44	0.425	1.22	0.86	−1.72	0.268
Ethnicity									
Afro-Ecuadorian	599 (25.7%)	1				1			
Non-Afro-Ecuadorian	1,733 (74.3%)	0.97	0.79	−1.20	0.774	0.84	0.64	−1.10	0.2
Education									
Illiterate	353 (15.1%)	1				1			
Primary	1,370 (58.8%)	1.17	0.88	−1.54	0.287	1.31	0.88	−1.93	0.183
Secondary	609 (26.1%)	1.34	0.99	−1.82	0.06	**1.70**	**1.12**	**−2.59**	**0.013**
Allergic symptoms									
No	2,204 (95.1%)	1				1			
Yes	113 (4.9%)	**1.62**	**1.12**	**−2.36**	**0.011**	1.43	0.86	−2.37	0.17
Allergen SPT+									
No	1,528 (75.2%)	1				1			
Yes	503 (24.8%)	**1.57**	**1.27**	**−1.93**	**<0.001**	**1.68**	**1.28**	**−2.21**	**<0.001**
Polysensitization									
0	1,528(75.2%)	1				1			
1	345(17.0%)	**1.33**	**1.03**	**−1.72**	**0.027**	**1.50**	**1.08**	**−2.07**	**0.014**
≥2	158(7.8%)	**2.11**	**1.55**	**−2.87**	**<0.001**	**2.09**	**1.40**	**−3.11**	**<0.001**
PATERNAL FACTORS									
Age (years)									
≤20	188 (8.1%)	1				1			
21–29	1,027 (44.0%)	0.82	0.59	−1.15	0.251	1.06	0.67	−1.70	0.800
≥30	1,117 (47.9%)	0.78	0.56	−1.08	0.138	0.99	0.62	−1.57	0.953
Ethnicity									
Afro-Ecuadorian	526 (23.3%)	1				1			
Non-Afro-Ecuadorian	1,734 (76.7%)	0.89	0.72	−1.10	0.279	0.81	0.61	−1.07	0.135
Education									
Illiterate	330 (15.5%)	1				1			
Primary	1,130 (53.0%)	0.96	0.73	−1.27	0.795	1.15	0.78	−1.68	0.486
Secondary	672 (31.5%)	1.00	0.75	−1.35	0.979	1.19	0.76	−1.73	0.508
Allergic symptoms									
No	2,074 (96.4%)	1				1			
Yes	77 (3.6%)	0.77	0.44	−1.35	0.363	0.79	0.38	−1.66	0.533
Allergen SPT+									
No	843 (70.8%)	1				1			
Yes	348 (29.2%)	1.27	0.97	−1.66	0.083	1.28	0.90	−1.84	0.173

(Continued)

TABLE 1 | Continued

Variable		SPT+ to any allergen				SPT+ to mite			
	***N* (%)**	**OR**	**95%CI**		***p*-value**	**OR**	**95%CI**		***p*-value**
Polysensitization									
0	843(70.8%)	1				1			
1	223(18.7%)	1.33	0.98	−1.82	0.070	1.31	0.86	−1.99	0.204
≥2	125(10.5%)	1.15	0.76	−1.74	0.495	1.23	0.72	−2.12	0.444
HOUSEHOLD FACTORS									
Residence									
Urban	1,638 (70.2%)	1				1			
Rural	694 (29.8%)	1.00	0.82	−1.23	0.979	**0.70**	**0.52**	**−0.93**	**0.013**
Monthly income									
<1 family basket	1,950(94.4%)	1				1			
>1 family basket	115(5.6%)	**1.48**	**1.02**	**−2.16**	**0.040**	**1.72**	**1.08**	**−2.74**	**0.024**
Material goods									
1–2	1,160 (49.7%)	1				1			
3–4	1,172 (50.3%)	1.04	0.86	−1.25	0.703	1.21	0.95	−1.54	0.132
HYGIENE FACTORS									
Birth order									
1st−2nd	1,158 (49.7%)	1				1			
3rd−4th	725 (31.1%)	0.92	0.75	−1.13	0.431	**0.75**	**0.57**	**−1.00**	**0.049**
≥5th	449 (19.2%)	0.85	0.66	−1.09	0.194	**0.68**	**0.48**	**−0.96**	**0.028**
Daycare to 36 m									
No	1,862 (82.6%)	1				1			
Yes	392 (17.4%)	1.07	0.84	−1.36	0.593	1.04	0.76	−1.44	0.804
Daycare (months)									
0	1,862 (82.6%)	1				1			
1–12	281 (12.5%)	1.20	0.92	−1.57	0.183	1.18	0.83	−1.68	0.365
>12	111 (4.9%)	0.76	0.47	−1.23	0.260	0.71	0.37	−1.37	0.311
Crowding (tv)									
No	NA	1				1			
Yes		0.87	0.75	−1.02	0.083	0.87	0.72	−1.04	0.118
Crowding at birth									
No	956 (41.0%)	1				1			
Yes	1,376 (59.0%)	0.95	0.78	−1.15	0.594	0.88	0.68	−1.12	0.292
House move (tv)									
No	NA	1				1			
Yes		1.14	0.99	−1.32	0.071	1.08	0.91	−1.27	0.390
House construction (tv)									
Traditional	NA	1				1			
Non-traditional		1.11	0.91	−1.36	0.313	1.14	0.89	−1.47	0.31
House construction at birth									
Traditional	598 (25.6%)	1				1			
Non-traditional	1,734 (74.4%)	1.10	0.88	−1.38	0.395	**1.42**	**1.04**	**−1.93**	**0.027**
Potable water at birth									
No	1,527 (65.5%)	1				1			
Yes	805 (34.5%)	1.10	0.91	−1.33	0.327	1.24	0.97	−1.60	0.087
Bathroom (tv)									
WC	NA	1				1			
Latrine		0.91	0.76	−1.10	0.342	0.83	0.66	−1.05	0.118
Bathroom at birth									
WC	694 (29.8%)	1				1			
Latrine	1,638 (70.2%)	0.85	0.70	−1.04	0.112	**0.71**	**0.55**	**−0.91**	**0.007**

(Continued)

TABLE 1 | Continued

Variable		SPT+ to any allergen				SPT+ to mite			
	***N* (%)**	**OR**	**95%CI**		***p*-value**	**OR**	**95%CI**		***p*-value**
Dog in house (tv)									
No	NA	1				1			
Yes		1.14	0.99	−1.31	0.074	1.06	0.90	−1.25	0.508
Dog in house at birth									
No	1,996 (85.6%)	1				1			
Yes	336 (14.4%)	0.97	0.75	−1.27	0.833	0.97	0.68	−1.38	0.86
Cat in house (tv)									
No	NA	1				1			
Yes		0.99	0.86	−1.15	0.937	0.98	0.83	−1.16	0.794
Cat in house at birth									
No	1,961 (84.1%)	1					1		
Yes	371 (15.9%)	1.02	0.79	−1.31	0.897	1.06	0.76	−1.47	0.725
Farm animals (tv)									
0	NA	1				1			
1–2		1.00	0.84	−1.19	0.972	0.93	0.76	−1.15	0.495
≥3		1.35	0.98	−1.86	0.066	1.24	0.85	−1.83	0.271
Farm animals at birth									
0	1,510 (64.7%)	1				1			
1–2	675 (29.0%)	0.91	0.73	−1.12	0.353	0.81	0.61	−1.07	0.134
≥3	147 (6.3%)	0.73	0.48	−1.12	0.149	0.68	0.38	−1.20	0.18
Agriculture (tv)									
No	NA	1				1			
Yes		0.98	0.83	−1.16	0.812	0.84	0.68	−1.03	0.097
Agriculture at birth									
No	1,121 (48.1%)	1				1			
Yes	1,211 (51.9%)	1.04	0.86	−1.25	0.679	0.95	0.74	−1.21	0.667
Insects at 8 years									
0	648(33.2%)	1				1			
1	758(38.8%)	1.04	0.82	−1.32	0.732	1.00	0.73	−1.36	0.985
≥2	547(28.0%)	1.25	0.97	−1.60	0.085	1.12	0.81	−1.55	0.486
Ticks in last 12 months									
No	1,488 (76.0%)	1				1			
Yes	470 (24.0%)	0.98	0.78	−1.24	0.871	0.81	0.59	−1.11	0.181
STH PARASITES									
Any infected in household									
No	771 (40.3%)	1				1			
Yes	1,144 (59.7%)	0.83	0.67	−1.02	0.070	**0.68**	**0.52**	**−0.89**	**0.005**
Father									
No	708 (73.1%)	1				1			
Yes	260 (26.9%)	0.88	0.73	−1.06	0.164	0.85	0.55	−1.31	0.454
Mother									
No	1,249 (53.9%)	1				1			
Yes	1,070 (46.1%)	0.90	0.65	−1.24	0.509	**0.72**	**0.56**	**−0.92**	**0.009**
Any STH in child before 24 m									
No	2,089(89.6%)	1				1			
Yes	243(10.4%)	0.85	0.61	−1.19	0.340	0.92	0.60	−1.41	0.710
STH child (tv)									
No	NA	1				1			
Yes		0.86	0.71	−1.10	0.110	**0.70**	**0.55**	**−0.89**	**0.004**

(Continued)

TABLE 1 | Continued

Variable		SPT+ to any allergen				SPT+ to mite			
	N (%)	OR	95%CI		*p*-value	OR	95%CI		*p*-value
Anthelmintics (to 24 m)									
No	1,506 (64.6%)	1				1			
Yes	826 (35.4%)	0.92	0.74	−1.15	0.465	0.94	0.72	−1.25	0.687
Anthelmintics (tv)									
No	NA	1				1			
Yes		1.18	0.93	−1.49	0.165	1.34	1.00	−1.79	0.051

Data are for 2,332 children with at least one evaluation for SPT. Odds ratios (ORs) and 95% confidence intervals (CI) were estimated by fitting age and age^2-adjusted population-average longitudinal models using generalized estimating equations. Longitudinal binary outcomes were defined by presence/absence of atopic outcomes in children followed up between 2 and 8 years of age. Models were fit under missing completely at random assumption for unobserved data points. Characteristics are at time of birth of child or time-varying over the course of follow-up. STH—soil-transmitted helminth infections. Anthelmintic treatments—number of maternal reports of at least one anthelmintic treatment during the previous year. Atopy was measured by allergen skin prick test reactivity to a panel of 9 relevant aeroallergens. Parental allergic symptoms—history of symptoms of asthma, rhinitis, and eczema. One family basket: a household income sufficient to meet the basic needs of 4 persons in 2008 was $480 monthly. Traditional building materials were wood and bamboo. Non-traditional materials were cement, block, and brick. Household overcrowding was defined as 3 or more people per sleeping room. House move represented a change in location of the child's household since the last follow-up evaluation. Agricultural exposures was defined by living on a farm or having at least weekly visits to a farm. Haematophagous insects represented presence of types of haematophagous insects (ticks, reduviid bugs, bedbugs, and lice) in the house over the previous year while ticks in the past 12 months represented the child having a documented tick bite during the previous year. Any infected with STH in the household represented any member of the child's household with a positive stool sample collected around the time of birth of the cohort child. Maternal and paternal atopy were to any allergen or mite allergens, as appropriate. Age effect per month: SPT+ to any allergen—age OR 1.058, 95% CI 1.043–1.072, P < 0.001; age^2 OR 0.9997, 95% CI 0.9996–0.9998, P < 0.001/SPT+ to mite -age OR 1.057, 95% CI 1.040–1.073, P < 0.001; age^2 OR 0.9996, 95% CI 0.9995–0.9998, P < 0.001. Missing data: maternal allergic symptoms (17) and atopy (301); paternal ethnicity (72), education (200), allergy (81), and atopy (1141); household income (267); daycare (78); haematophagous insects (379) and child exposure to ticks (374); maternal STH (13); paternal STH (1364); and household STH (417). P < 0.05 are shown in bold.

(unless otherwise specified) are shown in **Table 1**. Total numbers of anthelmintic treatments received by the 2,404 newborns recruited over up to 8 years of follow-up were: 0–1 (8.1%), 2–3 (44.1%), and ≥4 (47.8%) treatments. Parasite burdens with *A. lumbricoides* and *T. trichiura* were predominantly light with a minority having moderate or heavy infection intensities at any specific age between 7 months to 8 years (peak percentage for moderate−35% for *A. lumbricoides* at 30 months and 17% for *T. trichiura* at 24 months; heavy−9% for *A. lumbricoides* at 30 months and 2% for *T. trichiura at* 30 months). Proportions with allergic disease symptoms at 8 years among 1,971 children with available data were wheeze (6.6%), eczema (7.9%), and rhinitis (2%).

Patterns and Emergence of Allergic Sensitization in Children and Their Parents

Percentages of children with SPT+ at 8 years and their parents (2,031 mothers and 1,191 fathers) to the same 6 aeroallergens (*D. pteronyssinus/farinae*, American cockroach, dog, cat, fungi, and grass pollens) are shown in **Figure 2**. Fathers had the greatest rates of sensitization to any of these allergens (29.0%), followed by mothers (24.8%) and cohort children (13.8%). The dominant aeroallergens in this population were *Dermatophagoides* spp. and American cockroach (fathers 18.8 and 17.8%, respectively; mothers 15.7 and 14.7%; and children 10.7 and 5.3%). Allergic sensitization among parents to other aeroallergens were: grass pollens (fathers 5.4% and mothers 3.2%), fungi (5.6 and 2.7%), dogs (2.2 and 1.5%), cats (1.3 and 1.2%), and *A. tenuis* (2.6 and 1.5%). Rates of sensitization to allergens not included in **Figure 2** were to the mites *B. tropicalis* (fathers 6.6% and mothers 4.7%) and *C. arcuatus* (6.6 and 3.9%), and to the fungus *A. tenuis* (2.6 and 1.5%). Allergic sensitization to the 9 aero and food allergens tested in cohort children from 2 through to 8 years is shown in **Figure 3**: percentages of children with sensitization to any allergen was greatest at 8 years (14.8%) and was 13.8% to perennial and 0.5% to food allergens. Significant sensitization to individual allergen extracts (>2%) were only seen for *Dermatophagoides* spp. and cockroach: *Dermatophagoides* spp. sensitization was present at 2 years and increased rapidly while cockroach sensitization emerged later at 5 years. Proportions of children with SPT+ to any allergen but without mite sensitization were 50.8% at 2, 37.9% at 3, 41.1% at 5, and 27.4% at 8 years (equivalent proportions for mothers and fathers were 36.7 and 34.9%, respectively). Relatively few children had sensitization to food allergens with rates of ≤1.3% (to any of milk, egg, and peanut) at any of the observation times. Polysensitization (sensitization to more than one allergen) increased with age: 2 years (0.7%), 3 years (1.4%), 5 years (2.8%), and 8 years (4.2%). Rates of polysensitization in mothers and fathers were 7.9 and 10.4%, respectively.

Exposures Associated With Allergic Sensitization to Any Allergen During First 8 Years of Life

Longitudinal analyses showed that the risk of SPT+ to any allergen were strongly associated with age in a non-linear fashion (**Table 1** and **Figure 4**). Minimally age-adjusted population-averaged and multivariable associations between child, parental, household economic, and hygiene factors and development of SPT+ during the first 8 years of life are shown in **Table 1**. Age-adjusted models showed significant positive associations between SPT+ to any allergen and maternal allergic symptoms (OR 1.62, 95% CI 1.12–2.36) and SPT+ (OR 1.57, 95% CI 1.27–1.93) and having a household income >$470/month

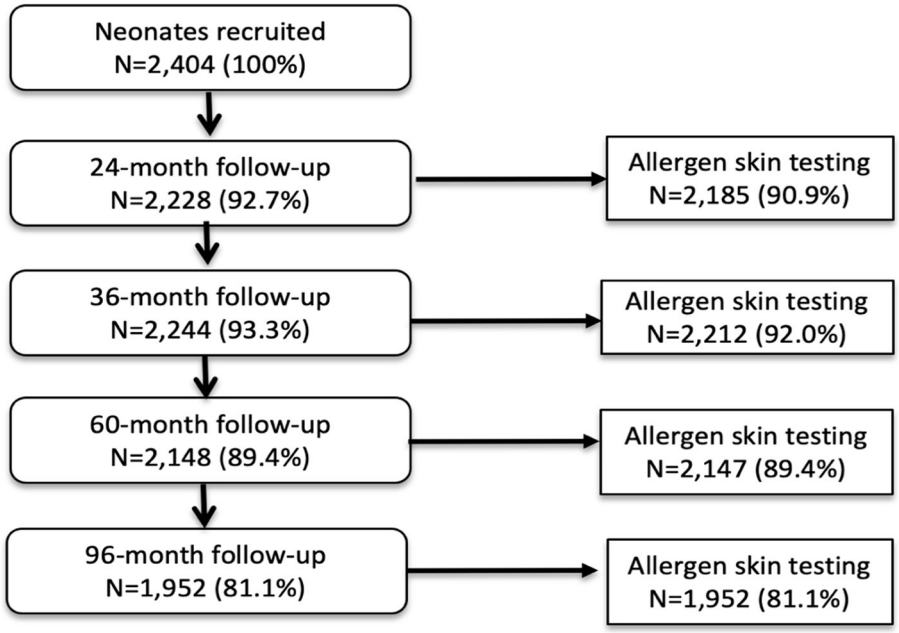

FIGURE 1 | Flow diagram to show follow-up of cohort to 8 years and allergen skin prick testing. Denominator for all proportions is 2,404. A child with any SPT result during follow-up was included in the longitudinal analysis.

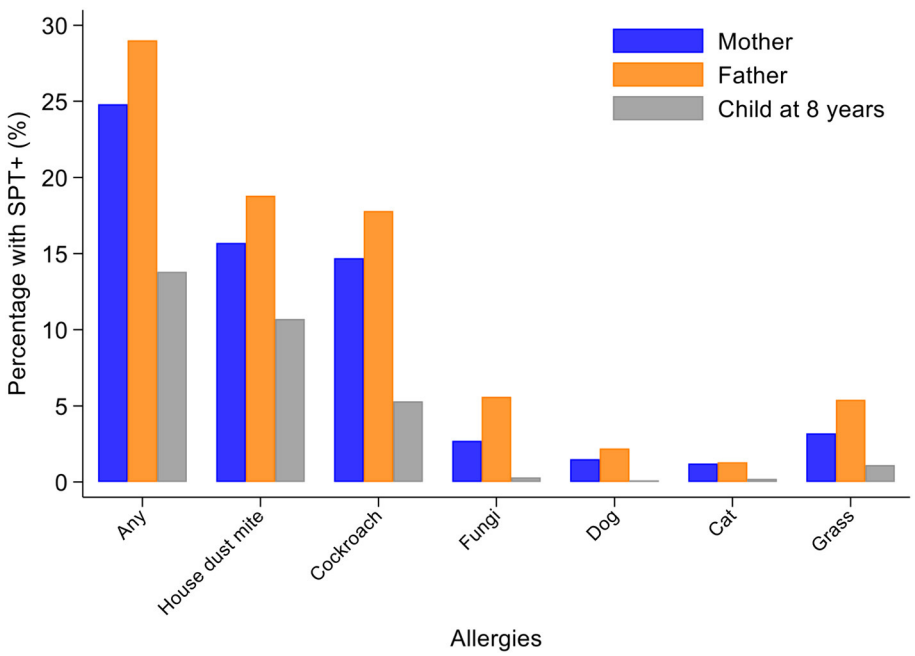

FIGURE 2 | Percentages of mothers, fathers, and cohort children (at 8 years) with positive skin prick tests (SPT+) to any allergen and individual allergen extracts. Data for Mothers ($n = 2,031$) (blue bars), fathers ($n = 1,191$) (orange bars), and children at 8 years ($n = 1,952$) (gray bars).

(equivalent to meeting a household's basic needs) (OR 1.48, 95% CI 1.02–2.16). An increasing number of maternal SPT+ reactions was associated with a greater risk of childhood SPT+ to any allergen (vs. SPT–: monosensitization, OR 1.33, 95% CI 1.03–1.72; polysensitization, OR 2.11, 95% CI 1.55–2.87) (**Table 1** and **Figure 5**).

In multivariable analyses (**Table 2**), childhood SPT+ to any allergen was significantly associated with maternal allergic

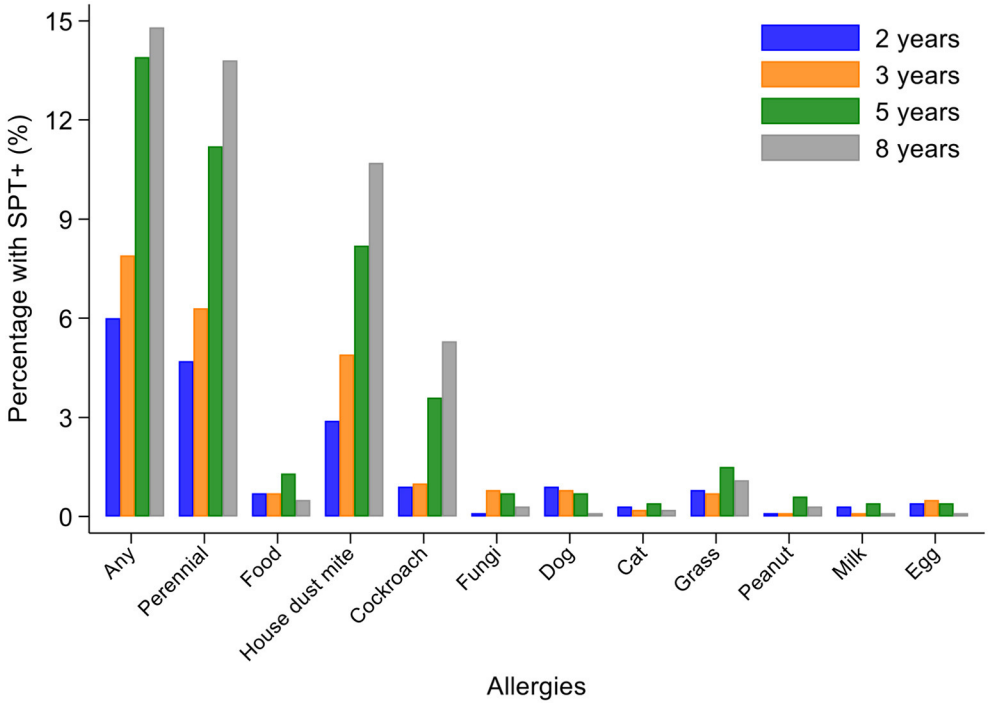

FIGURE 3 | Changes in allergic sensitization profiles between 2 and 8 years of age in cohort. Bars represent 2 (blue), 3 (orange), 5 (gray), and 8 (yellow) years. Perennial allergens included mite, cockroach, dog, and cat allergens.

symptoms (OR 1.54, 95% CI 1.03–2.29) and SPT+ with strong effects seen for more maternal SPT+ reactions (vs. SPT–: monosensitization, OR 1.35, 95% CI 1.04–1.73; polysensitization, OR 2.04, 95% CI 1.49–2.77) (**Figure 5**) while post-natal household overcrowding was inversely associated with SPT+ (OR 0.84, 95% CI 0.72–0.98).

Exposures Associated With Mite Sensitization During First 8 Years of Life

Mite sensitization was strongly associated with age (**Table 1** and **Figure 4**). Age-adjusted analyses showed significant positive associations for mite SPT+ with greater maternal education status (completed secondary vs. illiterate, OR 1.70, 95% CI 1.12–2.59), maternal mite sensitization (OR 1.68, 95% CI 1.28–2.21) and polysensitization (vs. SPT–, OR 2.09, 95% CI 1.40–3.11), having a greater household income (OR 1.72, 95% CI 1.08–2.74), and living in a household constructed with non-traditional materials (OR 1.42, 95% CI 1.04–1.93), while inverse associations were observed for greater period of breastfeeding (7–12 months vs. ≤6 months, OR 0.67, 95% CI 0.45–0.99), rural residence (OR 0.70, 95% CI 0.52–0.93), being lower in the birth order (vs. 1st−2nd: 3rd−4th, OR 0.75, 95% CI 0.57–1.00; ≥5th, OR 0.68, 95% CI 0.48–0.96), type of bathroom at time of birth (latrine vs. WC, OR 0.71, 95% CI 0.55–0.91), and STH infections (any household member with STH [OR 0.68, 95% CI 0.48–0.96] maternal STH [OR 0.72, 95% CI 0.56–0.92], and STH infections during childhood [OR 0.70, 95% CI 0.55–0.89]). Interestingly, there was some evidence that anthelmintic treatments given during childhood increased the risk of mite sensitization (OR 1.34, 95% CI 1.00–1.79).

In multivariable analyses (**Table 2**), mite sensitization during childhood remained significantly positively associated with greater number of maternal SPT+ reactions (vs. SPT–: monosensitization OR 1.64, 95% CI 1.17–2.29; polysensitization, OR 2.14, 95% CI 1.40–3.27) (**Figure 5**) and anthelmintic treatments received during childhood (OR 1.47, 95% CI 1.05–2.05) but inversely associated with rural residence (OR 0.69, 95% CI 0.50–0.94), birth order (3rd−4th and ≥5th vs. 1st−2nd, OR 0.71), agricultural exposures (OR 0.77, 95% CI 0.60–0.98), and STH infections acquired during childhood (OR 0.70, 95% CI 0.64–0.91). Maternal STH were not significantly associated with SPT to any allergen or to mite in the adjusted longitudinal analyses. Analysis of effects of parasite type in age-adjusted analyses showed a protective against mite sensitization of maternal and childhood *T. trichiura* (maternal—OR 0.63, 95% CI 0.44–0.90, $P = 0.012$; childhood—OR 0.58, 95% CI 0.35–0.98, $P = 0.042$) and childhood *A. lumbricoides* (0.74, 95% CI 0.55–0.99, $P = 0.042$; **Figures 6**, **7**). There was evidence for stronger protective effects against mite SPT+ of higher parasite burdens with *A. lumbricoides* and *T. trichiura* during childhood (i.e., as a time-varying exposure) although this was statistically significant only for *A. lumbricoides* (moderate-heavy vs. uninfected, OR 0.36, 95% CI 0.18–0.72) (**Table 3**).

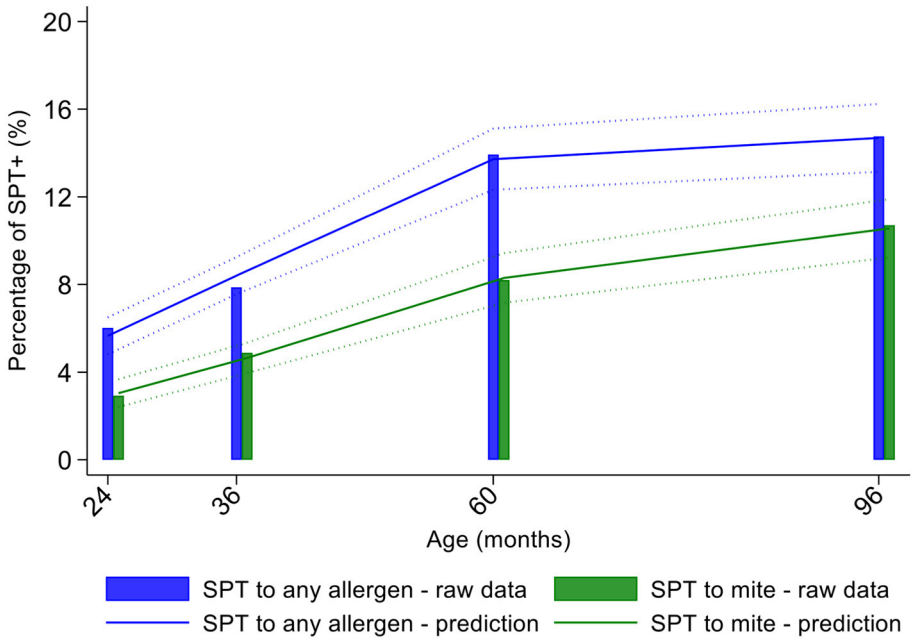

FIGURE 4 | Age-dependent proportions of cohort children with allergen skin prick test positivity (SPT+) to any allergen (blue) and to mite (green). Shown are predictions from population average longitudinal models (predictions shown by solid lines and 95% confidence intervals by dotted lines) against raw data percentages (bars).

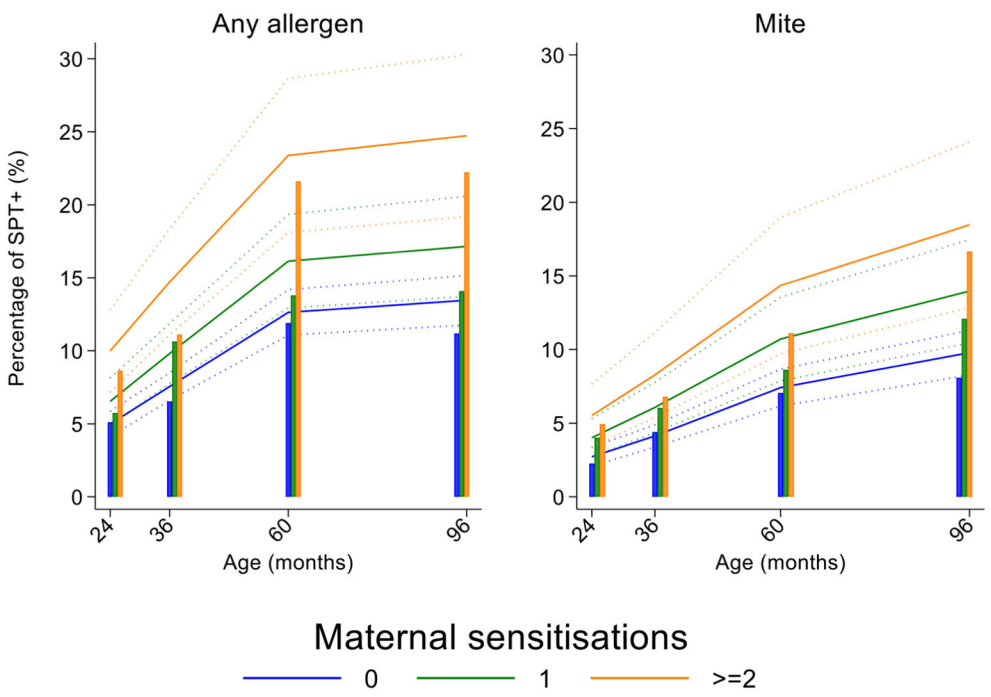

FIGURE 5 | Effects of number of maternal allergen skin prick test positive (SPT+) reactions on age-dependent proportions of cohort children with SPT+ to any allergen and to mite. Shown are predictions from population average longitudinal models (predictions shown by solid lines and 95% confidence intervals by dotted lines) against raw data percentages (bars) stratified by maternal sensitizations. Blue lines/bars—0 maternal SPT+ reactions; green lines/bars—1 SPT+ reaction; orange lines/bars—two or more SPT+ reactions.

TABLE 2 | Multivariable associations between risk of allergen skin prick test positivity (SPT+) to any and to mite allergens during first 8 years of life and child, parental, household, and hygiene factors.

Variable	SPT+ to any allergen				SPT+ to mite			
	OR	95%CI		*p*-value	OR	95%CI		*p*-value
MATERNAL FACTORS								
Allergic symptoms								
No	1							
Yes	1.54	1.03	−2.29	0.035				
Polysensitization								
0	1				1			
1	1.35	1.04	−1.73	0.022	1.64	1.17	−2.29	0.004
≥2	2.04	1.49	−2.77	<0.001	2.14	1.40	−3.27	<0.001
HOUSEHOLD FACTORS								
Residence								
Urban					1			
Rural					0.69	0.50	−0.94	0.019
HYGIENE FACTORS								
Birth order								
1st−2nd					1			
3rd−4th					0.71	0.52	−0.98	0.036
≥5th					0.71	0.49	−1.05	0.085
Crowding (tv)								
No	1							
Yes	0.84	0.72	−0.98	0.032				
Agriculture (tv)								
No					1			
Yes					0.77	0.60	−0.98	0.035
STH PARASITE								
STH child (tv)								
No					1			
Yes					0.70	0.64	−0.91	0.008
Anthelmintics (tv)								
No					1			
Yes					1.47	1.05	−2.05	0.024

Population average multivariable models were fitted to the binary longitudinal outcomes defined by presence/absence of SPT+ in children followed up between 2 and 8 years of age. Estimates were controlled for age and age^2. Age effects were non-linear ($p < 0.001$): Models were fit using generalized estimating equations under missing completely at random assumption for unobserved data points. OR, odds ratio; 95% CI, 95% confidence intervals; STH, soil-transmitted helminth; tv, time-varying covariates.

DISCUSSION

In the present analysis, we followed up a birth cohort to 8 years of age in a tropical coastal region of Ecuador to describe patterns and emergence of allergic sensitization in parents and offspring and identify antenatal and postnatal individual and environmental factors that might affect allergic sensitization. Significant proportions of parents had allergic sensitization, mainly to arthropod allergens from mites and cockroaches, while proportions of children with allergic sensitization increased progressively with age, initially to mite but later to cockroach. Rates of sensitization to pollens, pets, fungi, and foods, remained consistently low during childhood. Of the factors we were able to study as potentially associated with allergic sensitization in childhood, maternal atopy was the most important risk factor while protective effects were associated with rural residence, agricultural exposures, and childhood STH infections.

A strength of the study was the prospective design and high rates of adherence to follow-up to 8 years of age of a population-based sample. The longitudinal design allowed us to do a repeated-measures longitudinal analysis for the outcomes (measured at 4 time-points) that estimated age-dependent risk of SPT+ in childhood and took into account also time-varying changes in some key environmental exposures such as childhood STH infections. Longitudinal analyses are more informative because they predict the risk of SPT+ at each time point during follow-up, providing an overall age-dependent risk, and insights into the dynamics of SPT+ risk by age during childhood in a manner not possible when outcomes are evaluated at a single point in time. Key outcomes (SPT+) and exposures (STH infections) were measured objectively thus

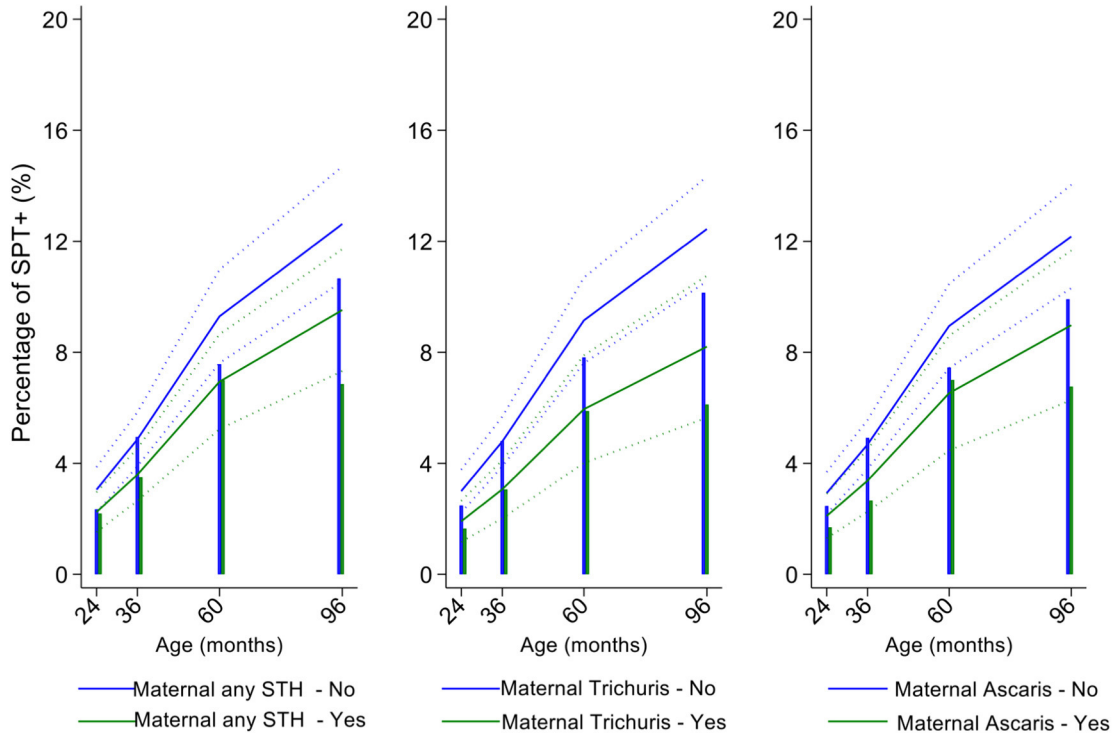

FIGURE 6 | Effect of maternal infections with any soil-transmitted helminth (STH) parasites or with specific STH species on age-dependent proportions of cohort children with allergen skin prick test positivity (SPT+) to mite. Shown are predictions from population average longitudinal models (predictions shown by solid lines and 95% confidence intervals by dotted lines) against raw data percentages (bars). Blue lines/bars—children of uninfected mothers; green lines/bars—children of infected mothers.

minimizing any potential systematic measurement bias. Recall bias is unlikely for risk factor data collected by questionnaire soon after birth for outcomes measured from 2 years of age. We were able collect data on a wide range of potential environmental exposures and potential confounders but cannot rule out potential confounding by unmeasured confounders such as maternal diet during pregnancy. We measured SPT+ to allergens selected as most relevant based on the findings of previous studies done in similar populations in coastal tropical Ecuador (22–24). It is quite possible, by inclusion of a limited panel of allergens, that we underestimated SPT+ in this population. For example, we did not measure SPT+ to *B. tropicalis* although a previous study of asthmatic children in the same province showed a much lower rate of SPT+ to *B. tropicalis* than to *Dermatophagoides* spp. (25). We were unable to measure SPT+ to pollens largely because of a lack of commercially available and geographically appropriate pollen allergen extracts for the South American region. It has been suggested that clinically relevant pollen sensitization may be less frequent in tropical and subtropical areas than more temperate climates (26). We measured allergen sensitization or atopy using allergen skin prick test positivity rather than measurement of specific IgE. The two measures of atopy are strongly correlated in high-income countries but tend to be dissociated in poorer populations living in low and middle-income countries (27–30), particularly where exposures to parasites such as the soil-transmitted helminth, *A. lumbricoides*, are common (6). Helminth parasite infections induce IgE responses to a wide variety of protein and carbohydrate molecules that cross-react extensively with allergens from multiple sources (31–33). Thus, IgE from helminth-infected individuals cross-react extensively with serological assays to detect allergens and allergen components resulting in common false-positive reactions (29, 30, 34). While it is clear that allergen-specific IgE at high titer from such individuals is strongly associated with allergic symptoms such as asthma (35), the relationship with low titer IgE where most of the IgE-allergen reactivity is seen, is less clear. For this reason, the use of allergen SPT+ is preferable for measuring allergic sensitization in populations living in tropical regions where helminth infections are endemic (30).

We used a birth cohort to define the emergence and development of allergic sensitization during early childhood to school age and measured atopy in parents to estimate rates of atopy in adults. Fathers had the highest rates of atopy (fathers 29% vs. mothers 24.8%) with similar rates of sensitization to mite (*D. pteronyssinus* and *D. farinae*, 18.8%) and American cockroach (17.8%), and lower rates of sensitization to pollens, fungi, and pets. Patterns were similar in mothers albeit at lower rates. Such patterns of allergic sensitization are consistent with previous

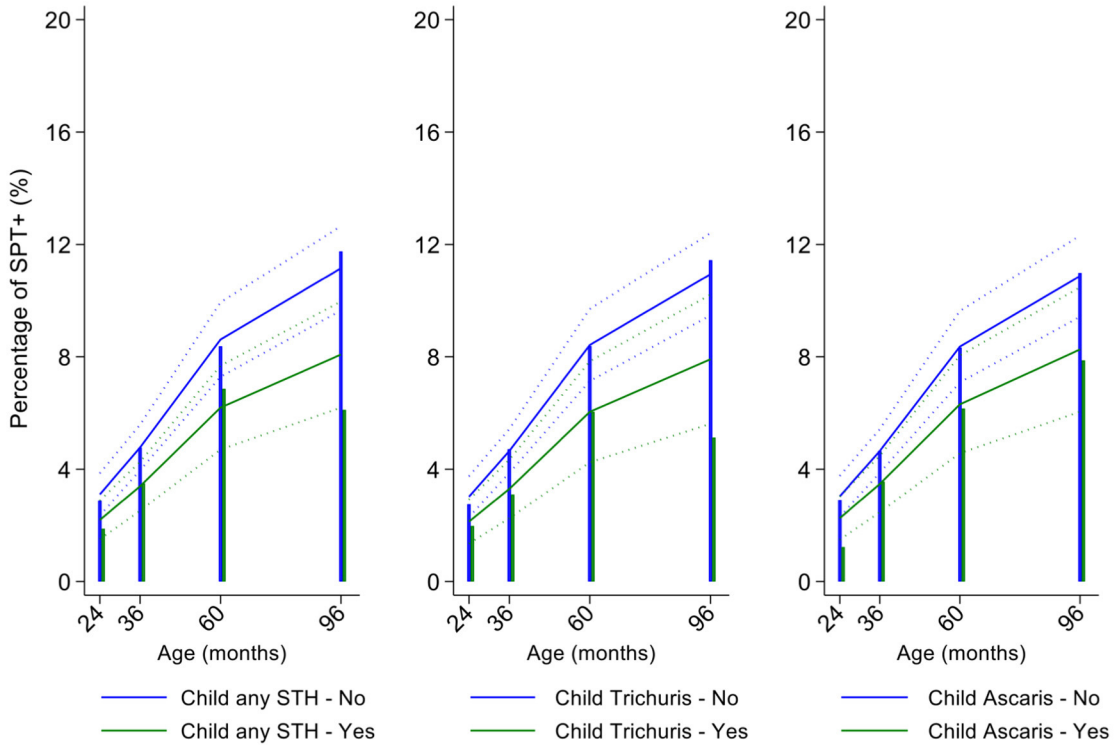

FIGURE 7 | Effect of childhood soil-transmitted helminth (STH) parasites or with specific STH species on age-dependent proportions of cohort children with allergen skin prick test positivity (SPT+) to mite. Shown are predictions from population average longitudinal models (predictions shown by solid lines and 95% confidence intervals by dotted lines) against raw data percentages (bars). Blue lines/bars—uninfected children; green lines/bars—infected children.

data from tropical urban environments (36–38). We saw much lower rates of sensitization (<7%) to the tropical mite, *Blomia tropicalis*, and the storage mite *Chortoglyphus arcuatus* compared to *Dermatophagoides* spp. *B. tropicalis* is an important source of allergens in tropical and subtropical settings and has been associated with respiratory allergies (6, 38), atopic dermatitis, and food allergies (39). Mite fauna in house dust samples from tropical settings including Ecuador are highly diverse (6, 40, 41) and vary geographically between such settings (6). The storage mite *C. arcuatus* has been reported in house dust samples from tropical Latin America (41, 42) where sensitization to this mite was observed in patients with asthma (41, 43) and allergic rhinitis (41). In this birth cohort, we observed early sensitization to *Dermatophagoides* spp. mites in 2.9% of children by 2 years of age, a rate that increased progressively to 10.7% at 8 years. Cockroach sensitization emerged at 5 years while sensitizations to other aeroallergens were negligible (i.e., <2%). The early emergence of mite sensitization in tropical settings could "drive" international comparisons of atopy, particularly if sensitization to other allergens emerged later in the life course. A bias toward sensitization to invertebrate allergens in early childhood in such a setting is perhaps not surprising given the tendency of children, living in conditions of poverty in precarious circumstances, to spend more time indoors in microenvironments that are increasingly sealed off from much of the animal and plant world and that, in the humidity and heat of the tropics, provide ideal conditions for the proliferation of mites and cockroaches that themselves produce allergens with a proclivity to become airborne. Polysensitization was relatively infrequent in both children (4.2% at 8 years) and parents (<11%). It should be remembered that this was an unselected population-based cohort with no enrichment for children (or parents) with allergic diseases and thus provides data on rates of sensitization in the general population.

A predominance of SPT+ to mite and cockroach allergens has been observed previously in cohorts of young children from tropical regions: (1) a study of 878 children aged 3 years in Ethiopia observed 8.7% positivity to any allergen (5.6% mite and 4.2% cockroach) (44); (2) a study of 132 young Indonesian children showed 19% had positive skin tests to any allergen at 4 years (10% cockroach and 7% mite) while very few (0.8%) had positive tests to food allergens (45); (3) a study of 1,170 children followed up to 9 years in Uganda with a subsample of 569 children tested at 3 years showed a positivity at 3 years of 18% to any allergen (*Dermatophagoides* spp. 11%, *Blomia tropicalis* 12%) and at 9 years of 25% to any allergen (*Dermatophagoides* spp. 18%, *B. tropicalis* 15%), and cockroach 12% with low positivity rates [<1.5%] to cat, molds, pollens, and food allergens [peanut, egg, and milk] (7); a study of 244 children aged 2–3 years in Colombia showed SPT+ to any allergen to be 22% (12% *B.*

TABLE 3 | Multivariable associations between risk of allergen skin prick test positivity (SPT+) to any and to mite allergens during first 8 years of life and infection intensities with *A. lumbricoides* and *T. trichiura*.

STH parasite	SPT+ to any allergen			SPT+ to mite		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
A. lumbricoides						
Uninfected	1			**1**		
Light	0.87	0.67–1.14	0.319	0.80	0.58–1.10	0.176
Moderate/heavy	0.78	0.51–1.17	0.229	**0.36**	**0.18–0.72**	**0.004**
T. trichiura						
Uninfected	1			**1**		
Light	0.90	0.70–1.16	0.402	0.78	0.57–1.07	0.129
Moderate/heavy	1.07	0.61–1.91	0.807	0.38	0.13–1.11	0.077

Population average multivariable models were fitted to the binary longitudinal outcomes defined by presence/absence of SPT+ in children followed up between 2 and 8 years of age. Estimates were controlled for age and age^2. Age effects were non-linear ($p < 0.001$): STH, soil-transmitted helminth; tv, time-varying covariates. Models were fit using generalized estimating equations under missing completely at random assumption for unobserved data points, and adjusted for age, maternal allergic symptoms and polysensitization, area of residence, birth order, crowding (tv), agriculture exposure (tv), and anthelmintic treatment (tv), according to outcome as shown for multivariable models in ***Table 2****. OR, odds ratio; 95% CI, 95% confidence intervals. Infection intensity categories were: A. lumbricoides (light−1–4,999; moderate−5,000–49,999; and heavy−≥50,000 epg) and T. trichiura (light−1–999; moderate−1000–9,999; and heavy−≥10,000 epg). P < 0.05 are shown in bold.*

tropicalis, 4% *D. pteronyssinus*, 2% cockroach, 5% cat, and 4% dog) while at 6 years SPT+ was 9% to *B. tropicalis*, 8% to *D. pteronyssinus*, 5% to cockroach, and <1.2% to cat and dog (8). As for the present study, the latter two studies showed the early emergence of mite sensitization and a low rate of sensitization to food allergens. There are relatively few studies of food allergy from tropical and sub-tropical regions of low and middle-income countries (LMICs) and there is a perception that food allergy is less frequent in LMICs compared to high-income countries, although current data are considered inadequate for systematic reviews of food allergy risk in LMICs (46, 47).

Previous cross-sectional studies done in pre-school and school age children in Ecuador have shown the following: (1) analysis of urban and rural schoolchildren in tropical regions of Esmeraldas Province showed prevalence of SPT+ to any allergen of 10.0% (7.6% to mite and 2.2% to cockroach) in urban and 12.5% (6.8% to mite and 4.5% to cockroach) in rural areas (24); (2) analyses of rural school children in sub-tropical and tropical areas of Pichincha Province showed prevalence of SPT+ to any allergen of 20–24.0% (mite, 9.3–8.0%; cockroach 9.4–15%) in different samples (22, 23); (3) analysis of urban and rural schoolchildren in highland Ecuador (altitude >1,500 m) showed SPT+ to any allergen of 52.6% (42.1% to mite and 19.4% to cockroach) in urban and 55.9% (43.4% to mite and 28.4% to cockroach) in rural samples (48); (4) analysis of pre-school children aged 3–5 years in city of Cuenca in a highland region (>2,000 m altitude) showed SPT+ to any allergen of 33.5% (24.3% to mite and 2.6% to cockroach) (49); and analysis of schoolchildren in Quito in a highland region (altitude 2,800 m) aged 6–21 years showed SPT+ to any allergen of 34.4% (34.1% to mite) (50). National studies in samples of children have shown, therefore, a predominance of positivity to mite and cockroach allergens using highly standardized extracts from reputable suppliers (ALK, Greer, and Leti) indicating the importance of sensitization to arthropod allergens. Further, SPT+ was much greater in higher altitude areas of the country (i.e., >1,500 m where climate can vary from sub-tropical to more temperate) than tropical regions of the country despite climatic conditions in tropical areas favoring greater exposures to arthropod-derived allergens. However, significant densities of mites and concentrations of mite allergens have been observed in highland settings such as Quito where humidity is sufficient to support mite growth (41). Such geographic differences in rates of SPT+ are unlikely to be explained by regional differences in ethnicity given a greater level of African genetic admixture in coastal populations that might increase risk (51, 52). A more plausible explanation is differences in key allergy-modifying environmental factors. An exposure that is consistently greater in tropical and sub-tropical regions of the country is STH parasite infections that thrive in the warm and moist conditions in tropical areas of the country (53). STH infections have been shown consistently to be inversely associated with SPT+ in different populations in tropical and sub-tropical regions of coastal Ecuador (adjusted ORs varying 0.62–0.78 between different studies) (22–24). These observations point to the fact that socio-environmental factors are likely to be more important risk factors for allergic sensitization than allergen-specific factors such as dosage or timing of exposure.

In this analysis, we focussed largely on the role of factors relating to poor hygiene as potential determinants of the expression of allergic sensitization. The only factor that was significantly associated with a reduced risk of SPT+ to any allergen during the first 8 years of life was household overcrowding as observed previously in a cross-sectional analysis in schoolchildren from a neighboring district (24). Overcrowding could mediate a protective effect against allergic sensitization through increased risk of childhood infections or a more diverse microbiome (54). Environmental factors protective against mite sensitization in the longitudinal analyses included rural residence, birth order, agricultural exposures (defined by living on farm or at least weekly visits to a farm), and childhood infections with STH. Rural residence and farming exposures have been consistently shown to protect against allergic sensitization

in a wide variety of settings (55) and may mediate their effects through exposure to more biodiverse environments that provide strong immune regulatory signals (56) during the first 1,000 days of life. The effect of agricultural exposures was dependent on the presence of the exposure during childhood (i.e., time-varying variable) indicating active modulation of SPT+.

Similarly, childhood STH were significantly protective when present early in the life course with stronger effects observed for *T. trichiura* than for *A. lumbricoides*. These results are consistent with a previous analysis from this cohort of the effects of early life STH exposures on the presence of SPT+ to any allergen at 8 years of age (i.e., an analysis that ignored the effects of age on the acquisition of SPT+) in which protective effects were observed among children of STH-infected mothers. In the previous analysis, strongest protective effects against SPT+ at 8 years were seen among children with STH infections during the first 5 years of life who also had infected mothers (11). Here, using an analysis that estimated age-dependent risk of SPT+ rather than at a single point in time, we observed a significant protective effect of maternal infections with *T. trichiura* against mite SPT+ over the first 8 years of life. Further, we observed an effect of STH infections acquired during childhood (i.e., as a time-varying exposure) on mite SPT+ with the strongest protective effects being present in children acquiring *T. trichiura* infections who also had infected mothers. These findings could indicate evidence for an antenatal effect of maternal *T. trichiura* in reducing age-dependent risk of mite SPT+ but which required infections during childhood to maintain the effect. Potentially, maternal *T. trichiura* infections could induce *in utero* modulation of the Th2 immune response of the developing fetus such that the capacity for allergic sensitization in offspring is reduced with infections acquired during post-natal life reinforcing such modulatory effects. We have shown previously that maternal STH can sensitize the fetus to STH antigens (57) and the type of sensitization induced (e.g., for greater immune reactivity or tolerization) is likely determined by the nature of the maternal immune response to that parasite (45, 58). Detection of a specific effect on mite SPT+ may relate to the fact that this is the dominant sensitizing allergen in early childhood in this population and to the extensive immunological cross-reactivity between STH and mite allergens (e.g., tropomyosin, paramyosin, and glutathione-S-transferase) (6) that could cross-modulate parasite-mite allergen-induced immediate hypersensitivity responses (32).

Anthelmintic treatments given during childhood (i.e., a time-varying exposure) increased the risk of mite sensitization supporting a role for childhood STH infections, acquired in early childhood, in the active suppression of mite SPT+. We have shown previously: (1) in a cluster-randomized trial of albendazole given every 2 months to schoolchildren over a period of 12 months that anthelmintic treatment had no effect on SPT+ prevalence (23); and (2) in an observational study of the impact of a mass drug administration programme using the broad-spectrum anthelmintic drug ivermectin to eliminate onchocerciasis, that children living in intervention communities had a greater prevalence of SPT+, an effect that was explained best by a lower prevalence of *T. trichiura* (59). In the latter study, anthelmintic treatments had been started before the study children were born in intervention communities implying that a reduction in maternal STH infections or transmission in early life (but not at school-age) are critical for mediating the protective effects of STH against SPT+.

We observed a strong association between maternal but not paternal atopy on the development of allergic sensitization during childhood with stronger maternal effects observed with greater number of sensitizing allergens. Previous studies have shown parental history of atopic diseases to be a risk factor for allergic outcomes in offspring (60, 61). There are more limited published data on effects of parental allergic sensitization on sensitization in offspring. A study in Germany showed that maternal but not paternal SPT+ was associated with allergic sensitization in children aged 7–16 years (62). Maternal SPT+ could increase the risk of atopy in offspring through increased genetic risk, intra-uterine factors, and other maternal factors such as diet. Although heritability of allergen SPT+ has been estimated at 35%, findings of genome-wide association studies for this trait showed no specific associations and indicated that genetic effects were likely mediated through a large number of different genes each contributing only a small risk (63). The effect of maternal but not paternal atopy is less suggestive of genetic or shared environment than specific intra-uterine effects. Children can be sensitized to allergens *in utero* (64) and our data suggest that maternal SPT+, particularly multiple sensitizations (taken as a measure of greater propensity to atopy), may alter how a child's immune response is programmed to react to allergens during the process of immune development and maturation in early childhood. Such effects could also be mediated through epigenetic mechanisms induced by environmental triggers (e.g., maternal nutrition) occurring during limited time frames *in utero* and early post-natal life (65).

In conclusion, there are limited data from birth cohorts in populations in tropical regions of low and middle-income countries studying the natural history of allergen sensitization and the individual and environmental risk factors for sensitization early in the life course. Here, we followed up a birth cohort to 8 years of age showing that the dominant sensitizing allergens present in this tropical setting are those relating to arthropods, specifically mite (more *D. pteronyssinus* than *B. tropicalis*) and cockroach, and mite sensitization emerges earliest and predominates to school age. Our data showed a role for both antenatal and post-natal factors in determining the emergence of allergic sensitization in offspring. Maternal SPT+ increased a child's risk of SPT+ to any allergen indicating the importance of *in utero* factors in determining a child's long-term risk of atopy. Childhood STH infections and agricultural exposures played an important role in modifying the risk of mite sensitization post-natally even after controlling for maternal atopy. Our data were from a population-based sample and the findings are generalizable to similar populations of children living in rural settings in tropical areas of Latin America. Future prospective studies in tropical LMIC settings could usefully explore the causal link between the acquisition of allergen sensitization and the development of allergic diseases during childhood and how individual and environmental factors modify this link.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committees of Hospital Pedro Vicente Maldonado, Pedro Vicente Maldonado, Pichincha Providence; and Universidad San Francisco de Quito, Quito, Ecuador. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

PC conceived, designed, supervised the study, and drafted the manuscript. MB and DS provided important input to study design and conduct. MC and MV were responsible for data collection. IC did the statistical analysis. All authors reviewed the manuscript critically before submission.

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Risk Factors Associated With Health Care Utilization in Preschool Recurrent Wheezers in a Tropical Environment

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Introduction: The severity of wheezing episodes is related with the need for health services, but the factors associated with health care utilization in preschool recurrent wheezers in underdeveloped regions are unclear.

Objective: To evaluate the factors associated with health care utilization in preschool recurrent wheezers in Cartagena, Colombia.

Methods: One hundred twenty-seven recurrent wheezers (age 2–6 years old) who were admitted to the emergency room (ER) due to wheezing in a Pediatric reference hospital in Cartagena were included. Children were evaluated by means of questionnaires and classified according to the number of ER visits, need for hospitalization and history of intensive care unit (ICU) admission due to wheezing within the last year. Total serum IgE and specific IgE to house dust mite allergens (HDM) were measured by ImmunoCAP® and allergen sensitization was evaluated by skin prick tests (SPT).

Results: The maternal report of nocturnal cough without fever in their children increased the risk to have ≥5 ER visits in the last year due to wheezing. The use of montelukast was negatively associated with hospitalization, while a history of pneumonia and lack of tap water, increased the risk of hospitalization due to wheezing. A history of bronchiolitis, family history of asthma, cohabiting with two or more siblings, passive exposure to smoke and lack of sewage facilities increased the risk of ICU admission due to wheezing. The presence of atopy evaluated by SPT reactivity, total IgE levels or specific IgE to HDM were not associated with health care utilization. We also found that seroprevalence of positive IgE (≥0.35 kU/L) was 27% to *B. tropicalis* and 20.3% to *D. pteronyssinus* but the prevalence of positive IgE sensitization to these allergens was below 2% and 8% when evaluated by SPT, respectively.

Conclusions: Poverty indicators are associated with ICU admission in a group of preschool recurrent wheezers and should be considered as aggravating factors for

wheezing. These factors must be systematically assessed in the medical approach in underdeveloped regions in the tropics. Nocturnal cough without fever is a symptom associated with frequent ER visits while atopy was not associated with health care utilization in preschool recurrent wheezers.

Keywords: atopy, childhood, emergency room visit, wheezing, preschool, tropic

INTRODUCTION

Wheezing is one of the most common respiratory symptoms in preschool children, and it has a myriad of causes and various management strategies. About 40% of children have at least one wheezing episode before the age 6 years (1–4), and although most will overcome them over time without consequences, about one third of wheezers will develop asthma later in life (3, 4). Several studies conducted in temperate/industrialized regions classify wheezing into several phenotypes, but few patients can specifically be assigned to one of them (5–9). Unfortunately, these classifications are not useful for identifying the severity or for guiding management (6, 10).

There is no consensus on the definition of wheezing severity in preschool children. As an attempt to define severity in this age group, the Spanish Guideline for Asthma Management (GEMA, for its Spanish initials) makes an important statement about the nature of asthma in infants, suggesting that it is an episodic disease with asymptomatic periods between crises, and for that reason, other classifications based on adult asthma cannot be applied in children (11). In accordance with GEMA, the Japanese guideline of childhood asthma uses the frequency of symptoms to classify asthma severity in preschool children (and the need of controller medication) compared to adults (12). Other guidelines, such as the Expert Panel Report 3 (EPR3) from the National Heart, Lung, and Blood Institute, have used the need for hospitalization, ICU admission (13), and use of oral/parenteral corticosteroids over the previous year (14) as proxies for the risk aspect of severity.

It has been recognized that in underdeveloped tropical regions, the wheezing prevalence can be similar or even higher than those reported in developed, industrialized regions (15–19). This predisposes the population to a high level of morbidity, especially in preschool children (20). In Colombia, the prevalence of asthma in the general population is 12%. In children below 4 years of age, it reaches 19% (21). In this age group, the clinical patterns of wheezing episodes are typically exacerbated by acute respiratory infections. The severity of these episodes is presumed to be higher compared to those in temperate industrialized settings (22) possibly due to limited access to appropriate treatment and exposure to noxious environments in underdeveloped urban areas (16, 23). This is reflected in an increased number of ER visits, hospitalizations, and ICU admissions.

Some studies have analyzed the environmental risk factors associated with recurrent wheezing in populations of underdeveloped tropical regions (16, 24–26) but few have analyzed the relationship between environmental factors and health care utilization (27). A prospective birth study in an underdeveloped urban community from Cartagena reported a prevalence of recurrent wheezing of 14.2% during the first 2 years of life (24). In the present study, we aim to identify factors associated with health care utilization in preschool children with recurrent wheezing in Cartagena, Colombia.

METHODS

Study Population

A cross-sectional study was designed. One hundred twenty-seven children, between 2 and 6 years old, were recruited while attending the ER department at Hospital Infantil Napoleon Franco Pareja, a third level, pediatric reference hospital in Cartagena, Colombia. For eligibility, individuals must have had a history of at least three broncho-obstructive episodes in their life and had to experience a physician-confirmed wheezing episode that was improved with a short-acting bronchodilator during their ER visit (**Figure 1**). Children with other diagnoses or comorbidities that impaired lung function (cystic fibrosis, broncho-pulmonary dysplasia, airway malformations, or cardiac or neurologic abnormalities) were excluded. Questionnaires assessing demographic and environmental risk factors were administered by a trained physician to the accompanying parent, together with a clinical history and a physical examination. All the children were invited to return 2 weeks after discharge from the ER or hospitalization for blood sampling and SPT. This study was approved by the ethical committee of the Hospital Infantil Napoleon Franco Pareja (Act. 8-16/03/8), the parents provided written informed consent to participate for all the children and patient anonymity was preserved.

Definitions for Health Care Utilization

Three indicators were considered to evaluate health care utilization during wheezing episodes in this population: the number of ER visits during the previous year due to wheezing, a history of hospitalization due to wheezing during the previous year, and a history of admission to an ICU due to wheezing. Considering the sample size, we classified the frequency of ER visits into <5 and ≥ 5 episodes, so the groups "frequent episodes" and "persistent wheezers" according to GEMA (11), were merged here into one group ($n = 42$) and analyzed in all subsequent analyses. These indicators were reported by the parents when interrogated using questionnaires at the ER.

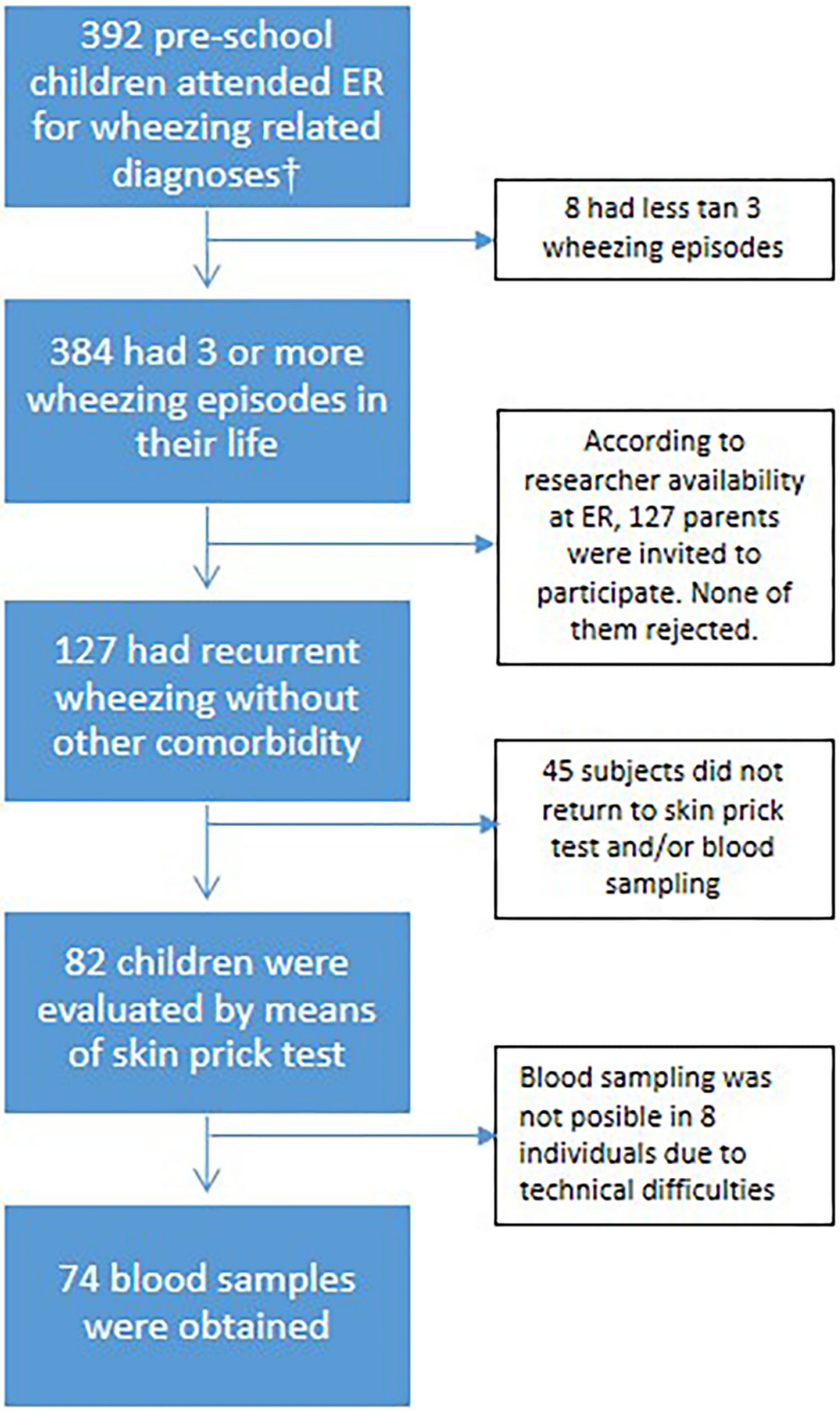

FIGURE 1 | Flowchart of participant's selection process. † The admission codes according to the International Statistical Classification of Diseases and Related Health Problems (ICD-10) were: J40X, J42X, J441, J450, J451, J458, J459, J46X, and J219.

Sociodemographic and Clinical Indicators

Previous validated questionnaires (24) were used to document sociodemographic conditions (age, gender, strata, housing conditions, siblings, pets, environmental exposures), details of the wheezing phenotype (age of onset, number of ER visits, hospitalizations, and ICU admissions, and use of medications),

personal history of allergic diseases (asthma, rhinitis or eczema), family history of allergic diseases, perinatal conditions, and personal history of infectious diseases. Socioeconomic strata in Cartagena are classified from 1 to 6, according to poverty indicators assessed by the municipality. Overcrowding was defined in accordance with the World Health Organization as 2.5 or more people sleeping in the same room. Since the definition of overcrowding varies throughout the literature and it is typically defined as the ratio of people per room in a house (WHO Housing and health guidelines. Geneva: World Health Organization; 2018). We here defined overcrowding as 2.5 or more people per room following the guide of the Economic Commission for Latin America and the Caribbean CELADE (http://hdl.handle.net/11362/9781). Children under 1 year of age were not included in this count, but older ones were.

Allergic Phenotypes

The personal history of allergic diseases was based on a self-report of physician-diagnosed asthma, physician-diagnosed rhinitis, and physician-diagnosed eczema. However, the questionnaire also contained questions for identifying symptoms at the time of recruitment, based on those we defined to be current asthma as three or more lifetime broncho-obstructive episodes and at least one episode during the previous year; current rhinitis was defined as two or more symptoms (rhinorrhea, nasal obstruction, nasal itching, and/or sneezing) described by the ARIA (Allergic Rhinitis and its Impact on Asthma) guidelines, with their severity classified as intermittent, persistent, mild, or moderately severe (28). Eczema was defined according to the criteria of the UK working party (29).

Skin Prick Tests and IgE Measurements

A subgroup of 82 children returned 2 weeks after discharge from the ER for skin prick tests. Eighteen allergens were tested: *Dermatophagoides farinae* (m602), *Dermatophagoides pteronyssinus* (m601), *Blomia tropicalis* (m608), feather mix (me01), *Aspergillus fumigatus* (p902), wheat (f004), flower mix (mb58), dog (e802), egg yolk (f075), egg albumin (f001), *Alternaria alternata* (p901), cat (e801), soy (f014), milk (f002), *Periplaneta americana* (i703), peanut (f013), grass (mw57), and pork (f026) (Inmunotek, Madrid, Spain). Histamine phosphate (k200) was used as the positive control and glycerol (k100) as the negative control. A test was considered positive when there was a wheal diameter equal or above 3 mm than the negative control after 20 min. Blood samples were taken via antecubital phlebotomy in 74 children. The tubes were incubated at room temperature for 2 h, and the serum was collected after centrifugation at 3000 rpm for 10 min. The serum was stored at −20°C until use. Total IgE and sIgE levels to *Blomia tropicalis* (d201) and *Dermatophagoides pteronyssinus* (d1) were measured by means of ImmunoCAP® (Thermo Fisher, Uppsala, Sweden) at the Institute for Immunological Research of the University of Cartagena. Total IgE levels were reported in IU/ml. sIgE levels above 0.35 kU/L were considered positive for IgE sensitization.

Statistics

Proportions between groups were compared by the chi square and Fisher exact tests, depending on the number of observations. Since the IgE was non-normally distributed, comparisons with continuous variables were performed with the Mann-Whitney U or Kruskall Wallis tests, as appropriate. Correlations were calculated by means of the Spearman test. In an exploratory manner to identify potential factors associated with health care utilization, the Odds Ratio (OR) was calculated for those variables with different proportions ($p < 0.05$) for ER visits, hospitalization and ICU admission. Those variables were included in a logistic regression model for each outcome, adjusting for the effect of age and gender. A p-value below 0.05 was considered statistically significant. Statistical calculations were performed with IBM SPSS Statistics 20 and GraphPad Prism 5.0. The Euler diagram was plotted using the Euler package version 4.1.0 by Larsson, J. (2018) (https://cran.r-project.org/package=eulerr).

RESULTS

The demographic, clinical characteristics, and environmental exposures of all children are presented in **Table 1**. Most subjects belonged to the lowest socioeconomic strata in the city and had an onset of wheezing before 12 months. Sixty-seven percent of the children were classified as occasional episodic wheezers, according to GEMA (0–4 episodes/year). About half of them had been hospitalized at least once due to wheezing during the previous year, and 19.7% had been admitted to ICU during their lifetime due to wheezing. A previous physician's diagnosis of asthma was already determined in 68% of the participants, and about 40% were under treatment with beclomethasone. Current rhinitis symptoms were reported by half of the patients, although a physician's diagnosis of rhinitis was only reported in 16.5%. The distribution of risk factors and exposures according to the number of ER visits, hospitalization and ICU admission is presented in **Table 2**.

Factors Associated With the Frequency of ER Visits Due to Wheezing

Report of nocturnal cough without fever (OR 4.3, 95% CI 1.8–10.4, $p = 0.001$) and current persistent rhinitis (OR 2.66, 95% CI 1.02–6.9, $p = 0.04$) were more frequent in children with >5 ER visits in the previous year due to wheezing. Neither other allergic manifestation nor a parental history of asthma or rhinitis were associated with the number of ER visits. Perinatal factors, sociodemographic aspects, living conditions, and history of infectious diseases were not associated with the number of ER visits. Nocturnal cough without fever was the most significant factor associated with increased risk of >5 ER visits during the previous year due to wheezing even after adjustment by age and gender (**Table 3**).

Factors Associated With Hospitalization During the Previous Year Due to Wheezing

Children who were hospitalized were significantly younger than children who did not (median 35 months, IQR 25–48 vs.

TABLE 1 | Demographic, clinical characteristics, and environmental exposures of the study children ($n = 127$).

Age (months)	36 (30–48)
Gender (Male)	72 (56.7%)
Lowest socioeconomic strata (strata 1)[a]	75 (59.1%)
Health services utilization due to wheezing	
Number of ER visits during the previous year due to wheezing	4 (2–6)
Prevalence of the wheezing phenotype according to the GEMA guideline	
Occasional episodic (0–4 episodes/year)	85 (66.9%)
Frequent episodic (5–8 episodes/year)	24 (18.9%)
Persistent (>8 episodes/year)	18 (14.2%)
History of hospitalization during the previous year	64 (54%)
History of ICU admission during their lifetime	25 (19.7%)
Allergic manifestations	
Age at first wheezing episode (months)	7 (3–12)
Report of nocturnal cough without fever	76 (59.8%)
Report of cough while doing sports or exercise	17 (13.4%)
Physician-diagnosed asthma	87 (68.5%)
Current use of beclomethasone	51 (40.2%)
Current use of montelukast	39 (30.7%)
Current rhinitis	64 (50.4%)
Rhinitis phenotypes according to the ARIA guideline ($n = 64$)	
Persistent rhinitis	21 (32.8%)
Moderate to severe rhinitis	32 (50%)
Physician-diagnosed rhinitis	21 (16.5%)
Report of eczema symptoms in the previous 6 months	17 (13.4%)
Any positive SPT ($n = 82$)	24 (29%)
Positive SPT to HDM ($n = 82$)	8 (9.7%)
Positive sIgE to HDM ($n = 74$)	23 (31%)
Family history of allergic diseases	
Family history of asthma (mother, father, and/or sibling)	79 (62.2%)
Maternal asthma	23 (18.1%)
Paternal asthma	17 (13.4%)
Family history of rhinitis (mother, father, and/or sibling)	70 (55.1%)
Perinatal factors	
Preterm birth (<37 weeks of gestational age)	31 (24.4%)
Cesarean delivery	81 (63.8%)
Neonatal ICU stay	20 (15.7%)
Reported previous infectious diseases	
Bronchiolitis	54 (42.5%)
Pneumonia	61 (48%)
Pyoderma	18 (14.2%)
Urinary tract infection	25 (19.7%)
Expulsion of roundworm	32 (25.2%)
Living conditions	
Two or more siblings	41 (32.3%)
Overcrowding	30 (23.6%)
Passive exposure to smoke	19 (15%)
Trash burning at home	44 (34.6%)
Pet ownership	53 (41.7%)
dog	37 (29.1%)
cat	14 (11.8%)
Lack of tap water	17 (13.4%)
Lack of sewage facilities	35 (27.5%)

Continuous variables are in median (interquartile range) and categorical variables in percentage.

ER, emergency room; GEMA, Spanish Guideline for Asthma Management (GEMA, for its Spanish initials); ICU, intensive care unit; ARIA, Allergic Rhinitis and its Impact on Asthma guidelines; SPT, skin prick test; HDM, house dust mite; sIgE, specific IgE; [a]Socioeconomic strata in Cartagena are classified from 1 to 6, according to the residential property and defined by the municipality.

39 months, IQR 35–52, $p = 0.01$, respectively). A history of pneumonia (OR 2.22, 95% CI 1.09–4.5, $p = 0.026$) was more frequent in children who were hospitalized during the previous year due to wheezing. Regarding living conditions, lack of tap water was also more frequent in hospitalized children (OR 3.7, 95% CI 1.15–12.2, $p = 0.028$). By contrast, current use of montelukast (OR 0.3, 95% CI 0.13–0.68, $p < 0.01$) and a physician's diagnosis of rhinitis (OR 0.33, 95% CI 0.11–0.91, $p = 0.02$) were more frequently reported among children that have not being hospitalized due to wheezing. Among these variables, current use of montelukast and a history of pneumonia remained significant after adjustment by age and gender (**Table 4**).

Factors Associated With ICU Admission During Their Lifetime Due to Wheezing

Current use of beclomethasone (OR 0.31, 95% CI 0.12–0.8, $p = 0.01$) and a family history of rhinitis (OR 0.24, 95% CI 0.09–0.62, $p = 0.004$) were less frequent in the ICU admission group. On the other hand, a history of bronchiolitis (OR 3.7, 95% CI 1.4–9.4, $p = 0.006$) or history of maternal asthma (OR 2.9, 95% CI 1.01–8.3, $p = 0.05$) were more frequent in children with ICU admission. Regarding socioeconomic factors, co-habiting with two or more siblings (OR 4.3, 95% CI 1.7–10.9, $p = 0.002$), exposure to passive smoke at home (OR 3.8, 95% CI 1.3–11.09, $p = 0.01$) and lack of sewage facilities (OR 2.5, 95% CI 1.02–6.3, $p = 0.04$) were more prevalent in children with a history of ICU admission due to wheezing. Overcrowding was also associated with ICU admission (OR 2.7, 95% CI 1.07–6.9, $p = 0.03$). Most of these factors remained associated with ICU admission due to wheezing after adjustment by age and gender (**Table 5**).

IgE Sensitization and Health Care Utilization

We then analyzed the relationship between IgE sensitization to common allergens with the number of ER visits, hospitalization, or ICU admission due to wheezing in 82 children that returned 2 weeks after discharge from the ER (**Figure 1**). Skin prick tests (SPT) were performed on a battery of 18 allergens. The prevalence of sensitization to any of the allergens tested was 26.8% ($n = 22$). The pattern of sensitization is presented in **Figure 2**. House dust mite and cockroaches were the most frequent sensitizers in this population

TABLE 2 | Distribution of variables according health services utilization in preschool wheezers.

	ER visits during the previous year			Hospitalization during the previous year			ICU admission during their lifetime		
	≥5 ($n = 42$)	<5 ($n = 85$)	*P*	Yes ($n = 64$)	No ($n = 63$)	*p*	Yes ($n = 25$)	No ($n = 102$)	*p*
Age (months)	36 (31–47)	37 (29–52)	0.5	35 (25–48)	39 (35–52)	**0.012**	33 (24–42)	37 (33–50)	**0.027**
Gender (Male)	27 (64.3%)	45 (52.9%)	0.2	36 (56.2%)	36 (57.1%)	0.9	16 (64%)	56 (54.9%)	0.4
Lowest socioeconomic strata	22 (52.4%)	53 (62.4%)	0.2	41 (64.1%)	34 (54%)	0.2	19 (76%)	56 (54.9%)	0.055
Health services utilization due to wheezing									
# of ER during the previous year	–	–	–	4 (2.2–6)	4 (2–5)	0.3	4 (2–5.5)	4 (2–6)	0.4
≥5 ER visits during the previous year	–	–	–	23 (35.9%)	19 (30.2%)	0.4	8 (32%)	34 (33.3%)	0.8
Hospitalization during the previous year	23 (54.8%)	41 (48.2%)	0.4	–	–	–	17 (68%)	47 (46.1%)	0.05
ICU admission during their lifetime	8 (19%)	17 (20%)	0.8	17(26.6%)	8 (12.7%)	0.05	–	–	–
Allergic manifestations									
Age of first wheezing episode (months)	7 (3.5–12)	7 (3–14)	0.4	7.5 (3.2–12)	7 (3–14)	0.9	6 (1–12)	8 (3.7–12.5)	0.09
Nocturnal cough without fever	34 (81%)	42 (49.4)	**0.001**	40 (62.5%)	36 (57.1%)	0.5	12 (48%)	64 (62.7%)	0.1
Physician-diagnosed asthma	31 (73.8%)	57 (67.1%)	0.4	45 (70.3%)	43 (68.3%)	0.8	21 (84%)	67 (65.7%)	0.7
Current use of beclomethasone	27 (64.3%)	42 (49.4%)	0.1	33 (51.6%)	36 (57.1%)	0.5	8 (32%)	61 (59.8%)	**0.012**
Current use of montelukast	11 (26.2%)	28 (32.9%)	0.4	12 (18.8%)	27 (42.9%)	**0.003**	4 (16%)	35 (34.5%)	0.092
Current rhinitis	25 (59.5%)	39 (45.9%)	0.1	34 (53.1%)	30 (47.6%)	0.5	10 (40%)	54 (52.9%)	0.2
Current persistent rhinitis	11 (26.2%)	10 (11.8%)	**0.04**	13 (20,3%)	8 (12,7%)	0.3	2 (8%)	19 (18,6%)	0.2
Current moderate to severe rhinitis	8 (19%)	24 (28.2%)	0.2	16 (25%)	16 (25.4%)	0.9	5 (20%)	27 (26.5%)	0.5
Physician-diagnosed rhinitis	6 (14.3%)	15 (17.6%)	0.6	6 (9.4%)	15 (23.8%)	**0.029**	2 (8%)	19 (18.6%)	0.2
Report of eczema symptoms in the previous 6 months	5 (11.9%)	12 (14.1%)	0.7	10 (15.6%)	7 (11.1%)	0.4	4 (16%)	13 (12.7%)	0.6
Any positive SPT ($n = 82$)	12 (33.3%)	12 (26.21%)	0.4	12 (36.4%)	12 (24.5%)	0.2	3 (27.3%)	21 (29.6%)	0.8
Family history of allergic diseases									
Family history of asthma (mother, father, and/or sibling)	28 (66.7%)	51 (60%)	0.4	37 (57.8%)	42 (66.7%)	0.3	20 (80%)	59 (57.8%)	**0.041**
Family history of rhinitis (mother, father, and/or sibling)	24 (57.1%)	46 (54.1%)	0.7	31 (48.4%)	39 (61.9%)	0.1	7 (28%)	63 (61.8%)	**0.002**
Perinatal factors									
Preterm birth (<37 weeks of gestational age)	10 (23.8%)	21 (24.7%)	0.9	14 (21%)	17 (27%)	0.5	4 (16%)	27 (26.5%)	0.2
Cesarean delivery	23 (54.8%)	58 (68.2%)	0.1	41 (64.1%)	40 (63.5%)	0.9	17 (68%)	64 (62.7%)	0.6
Neonatal ICU stay	9 (21.4%)	11 (12.9%)	0.2	13 (20.3%)	7 (11.1%)	0.1	4 (16%)	16 (15.7%)	0.9
Reported previous infectious diseases									
Bronchiolitis	22 (52.4%)	32 (37.6%)	0.1	26 (40.6%)	28 (44.4%)	0.6	17 (68%)	37 (36.3%)	**0.004**
Pneumonia	21 (50%)	40 (47.1%)	0.7	37 (57.8%)	24 (38.1%)	**0.026**	16 (64%)	45 (44.1%)	0.075
Expulsion of roundworm	9 (21.4%)	23 (27.1%)	0.4	18 (28.1%)	14 (22.2%)	0.4	10 (40%)	22 (21.6%)	0.057
Living conditions									
Two or more siblings	30 (71.4%)	56 (65.9%)	0.5	25 (39.1%)	16 (25.4%)	0.1	15 (60%)	26 (25.5%)	**0.001**
Overcrowding	10 (23.8%)	20 (23.5%)	0.9	11 (17.2%)	19 (30.2%)	0.085	10 (40%)	20 (19.6%)	**0.031**
Passive exposure to smoke	5 (11.9%)	14 (16.5%)	0.6	9 (14.1%)	10 (15.9%)	0.7	8 (32%)	11 (10.8%)	**0.008**
Pet ownership	13 (31%)	40 (47.1%)	0.083	27 (42.2%)	26 (41.3%)	0.9	10 (40%)	43 (42.2%)	0.8
Lack of tap water	2 (4.8%)	15 (17.6%)	0.054	13 (20.3%)	4 (6.3%)	**0.021**	4 (16%)	13 (12.7%)	0.7
Lack of sewage facilities	9 (21.4%)	26 (30.6%)	0.2	22 (34.4%)	13 (20.6%)	0.083	11 (44%)	24 (23.5%)	**0.04**

Significant P values are highlighted in bold.

(9.7 and 5.6%, respectively). We found no differences in sensitization rates according to the number of ER visits, hospitalization, or ICU admission. Nevertheless, due to the low number of observations per group we cannot rule

TABLE 3 | Factors associated with more than five ER visits due to wheezing ($n = 127$).

Variable	<5 episodes n (%)	≥5 episodes n (%)	OR (95% CI) crude	OR (95% CI) adjusted by age and gender
Nocturnal cough without fever	41 (48.2)	34 (81)	4.56 (1.8–10.9) $p = 0.001$	4.5 (1.8–10.9) $p = 0.001$
Current persistent rhinitis	10 (11.8)	11 (26.2)	2.6 (1.02–6.9) $p = 0.04$	2.9 (1.1–7.8) $p = 0.03$

TABLE 4 | Factors associated with hospitalization in the previous year due to wheezing ($n = 127$).

Variable	No n (%)	Yes n (%)	OR (95% CI) crude	OR (95% CI) adjusted by age and gender
Use of montelukast	27 (42.9)	12(18.8)	0.30 (0.13–0.68) $p = 0.004$	0.30 (0.13–0.68) $p = 0.004$
Physician-diagnosed rhinitis	15 (23.8)	6 (9.4)	0.33 (0.1–0.9) $p = 0.034$	0.33 (0.11–0.9) $p = 0.038$
History of pneumonia	24 (38.1)	37(57.8)	2.22 (1.09–4.5) $p = 0.027$	2.1 (1.02–4.3) $p = 0.044$
Lack of tap water	4 (6.3)	13 (20.3)	3.7 (1.1–12.2) $p = 0.028$	3.5 (1.05–11.6) $p = 0.041$

out a contribution of atopy on health care utilization in recurrent wheezers.

We also measured total and specific IgE in 74 children in which a blood sample was obtained. Median levels of total serum IgE were 159 IU/ml (IQR 55–272 IU/ml) and 0.01 kU/L for *B. tropicalis* (IQR 0.002–0.63) and 0.02 kU/L for *D. pteronyssinus* (IQR 0.01–0.13). There was a direct and significant correlation between total IgE levels and the specific IgE levels to *B. tropicalis* (Spearman rho = 0.61, $p < 0.0001$) and *D. pteronyssinus* (Spearman rho = 0.53, $p < 0.0001$). The seroprevalence of positive IgE (≥0.35 kU/L) to *B. tropicalis* was 27% and to *D. pteronyssinus* was 20.3%. Interestingly, the frequency of IgE reactivity to these allergens was considerably higher than those observed by SPT (**Figure 2**). When analyzed as continuous variables, we found no difference in the total or the specific IgE levels according to the number of ER visits, hospitalizations, or ICU admission. The distribution of IgE levels according ER visits and the antecedent of hospitalization due to wheezing is presented in **Figures 3**, **4**, respectively. When stratifying the children with low and high total IgE levels according to the 75th percentile of the distribution we found no association with any of the outcomes. Likewise, a positive IgE result to *B. tropicalis* or *D. pteronyssinus* (>0.35 kU/L) analyzed as a categorical variable was not associated with health care utilization.

DISCUSSION

This study reveals the potential factors associated with the number of ER visits, hospitalization, and ICU admissions due to wheezing in preschool children in an underdeveloped urban tropical setting, and it is one of the few that reveals particular risk factors for health care utilization due to wheezing in the tropics. The results are representative of those preschool children, in the lowest socioeconomic strata, with recurrent wheezing who seek medical services in Cartagena due to an exacerbation of the disease and agree with previous associations between poverty and poor hygiene with recurrent wheezing (23, 26, 30).

We found an increased prevalence of nocturnal cough and current persistent rhinitis in children with five or more ER visits during the previous year due to wheezing. This association has not been evaluated in previous studies. The nocturnal pattern of asthma symptoms, like dry cough, has been related to multi-triggers preschool wheezers and allergic phenotypes (31, 32). Interestingly, in our study none of the atopic markers (SPT or sIgE) or a parental history of asthma/allergies were associated with the number of ER visits. Current persistent rhinitis in children of this cohort is most probably non-atopic since there was no association of current persistent rhinitis with total IgE levels or sensitization to HDM allergens (data not shown). This clinical variable should be evaluated in a larger sample size to define its association with the recurrent need of ER visits.

The use of montelukast was the most significant protective factor for hospitalization due to wheezing. Some studies in preschool wheezers have shown that montelukast was effective for reducing caregiver-observed wheezing, the need for salbutamol and acute exacerbations that required oral corticosteroids or hospitalization (33, 34). However, a recent meta-analysis did not demonstrate benefit of montelukast in preschoolers with recurrent wheezing, but it raised the need of studies that evaluate a montelukast responder phenotype (35). It is feasible that children with a diagnosis and who were using this medication were more likely to be properly followed up and had access to the health care system or may also reflect awareness of their parents of their respiratory symptoms. On the other hand, a history of pneumonia was a significant factor associated with an increased risk of hospitalization, suggesting

TABLE 5 | Factors associated with ICU admission due to wheezing ($n = 127$).

Variable	No ($n = 102$) n (%)	Yes ($n = 25$) n (%)	OR (95% CI) crude	OR (95% CI) adjusted by age and gender
Current use of beclomethasone	61 (59.8%)	8 (32%)	0.31 (0.12–0.8) $p = 0.015$	0.29 (0.11–0.77) $p = 0.012$
History of bronchiolitis	37 (36.3%)	17 (68%)	3.7 (1.4–9.4) $p = 0.006$	3.2 (1.2–8.5) $p = 0.015$
History of maternal asthma	15 (14.7%)	8 (32%)	2.7 (1.0–7.4) $p = 0.05$	3.42 (1.18–9.9) $p = 0.023$
Family history of rhinitis	63 (61.8%)	7 (28%)	0.24 (0.09–0.62) $p = 0.004$	0.24 (0.09–0.65) $p = 0.005$
Overcrowding	20 (19.6%)	10 (40%)	2.7 (1.07–6.9) $p = 0.036$	2.6 (1.02–6.8) $p = 0.045$
Cohabiting with two or more siblings	26 (25.5%)	15 (60%)	4.3 (1.7–10.9) $p = 0.002$	5.6 (2.1–15.2) $p = 0.001$
Exposure to passive smoke	11 (10.8%)	8 (32%)	3.8 (1.3–11.09) $p = 0.01$	4.8 (1.5–14.7) $p = 0.006$
Lack of sewage facilities	24 (23.5%)	11 (44%)	2.5 (1.02–6.3) $p = 0.04$	2.35 (0.92–5.9) $p = 0.07$
History of parasite expulsion	22 (21.6%)	10 (40%)	2.42 (0.95–6.13) $p = 0.062$	2.5 (0.97–6.5) $p = 0.057$

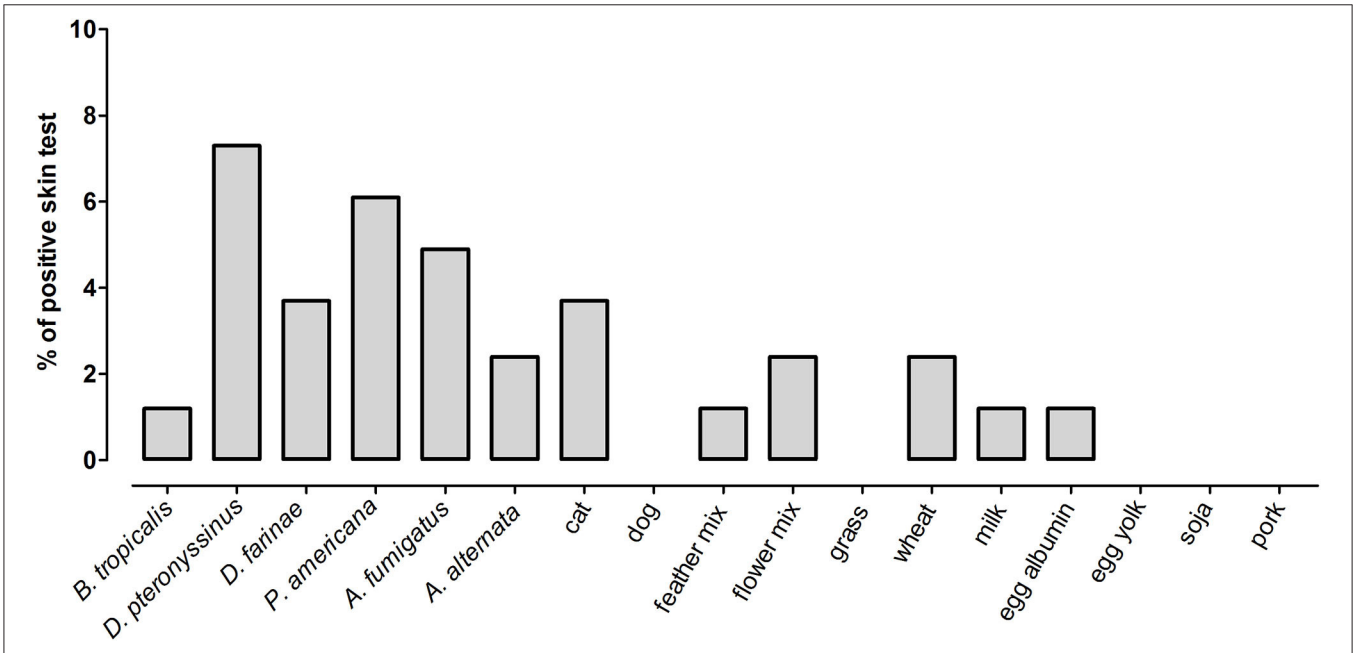

FIGURE 2 | Prevalence of positive skin tests to 18 allergens in a subset of wheezing children that returned 2 weeks after ER discharge ($n = 82$).

that lower respiratory infections may be critical as risk factor in this population. At the same time, some data suggests that pneumonia may be over diagnosed in children with asthma, especially in low-income countries (36, 37).

In this population, 25 children had a history of ICU admission due to wheezing. The most significant factor associated with protection was a family history of rhinitis. These findings could be explained by the fact that people with allergies are more aware of their respiratory symptoms and environmental triggers. Regarding living conditions, cohabiting with two or more siblings, passive exposure to smoke and lack of sewage facilities were the most significant factors associated with the increased risk of ICU admission. While several studies have previously shown that cohabiting with older siblings may protect against further development of allergic asthma, it is quite clear that in urbanized, underdeveloped regions this factor involve the increased number of people per household in the context of overcrowding. This unhygienic environment favors

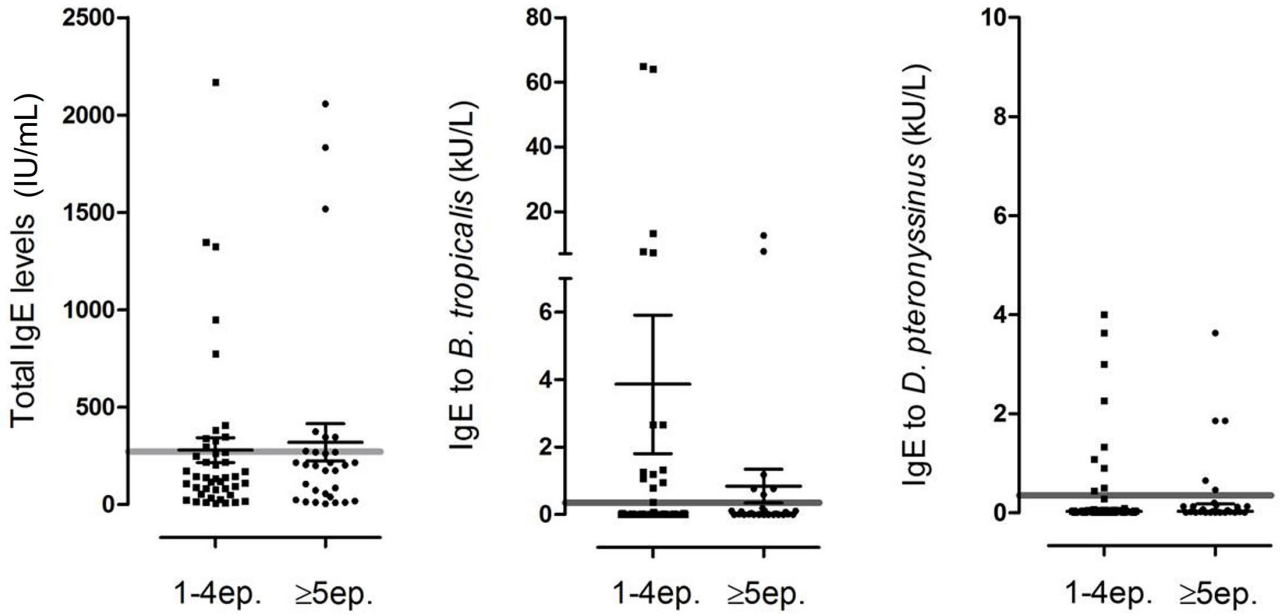

FIGURE 3 | Distribution of total and specific IgE levels in 74 children in which blood tests were available 2 weeks after discharge according to the number of ER visits due to wheezing. Each dot represents an individual, error bars indicate geometric mean and 95% confidence interval. Gray line indicates 300 IU/mL for total IgE and 0.35 kU/L for specific IgE.

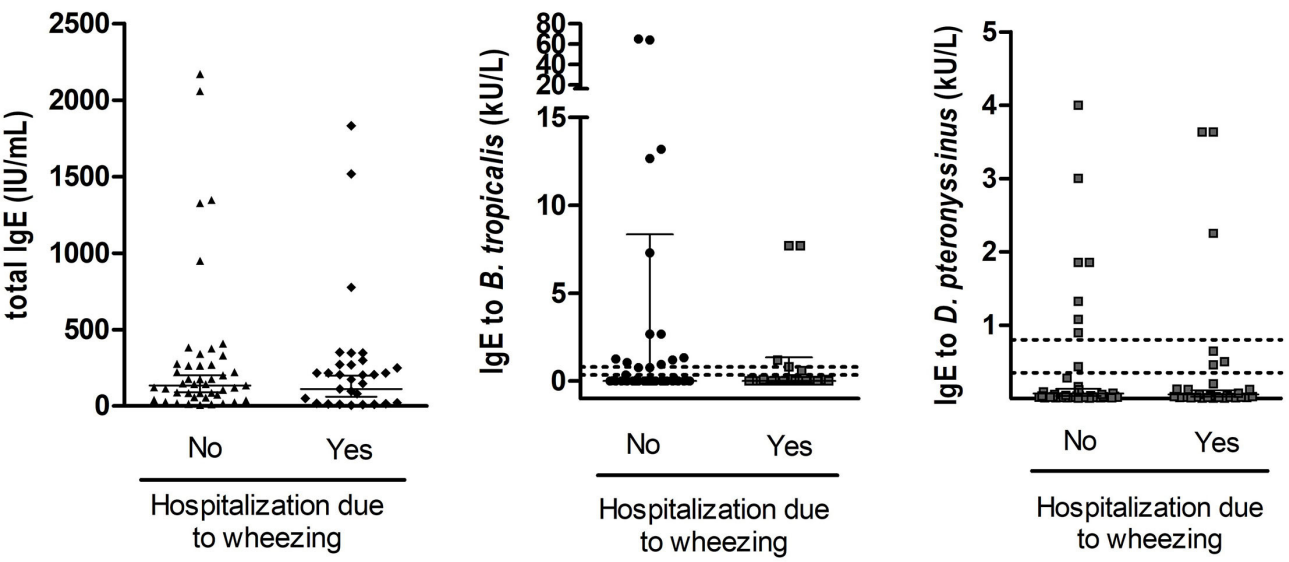

FIGURE 4 | Distribution of total and specific IgE levels in 74 children in which blood tests were available 2 weeks after discharge according to the antecedent of hospitalization in the previous year due to wheezing. Each dot represents an individual, error bars indicate geometric mean and 95% confidence interval. Discontinuous lines indicate the 0.35 and the 0.8 kU/l cut-off for positive IgE sensitization.

the development of severe communicable infections. Our results are in line with the observation that having older siblings is a risk factor for wheezing (38) and that low income and poverty (poor housing, low birth weight, and parasitic infections) are important risk factors for severe wheezing episodes in young children (39, 40). Further studies are needed to evaluate if the wheezing phenotype in this group reflects a persistent bacterial bronchitis, acute episodes of viral infections, or a different phenotype due to the scarce overlap between the children that had ever required ICU admission and those that had experienced recurrent ER visits (**Figure 5**). We also detected a non-significant tendency between a family history of asthma and an increased risk of

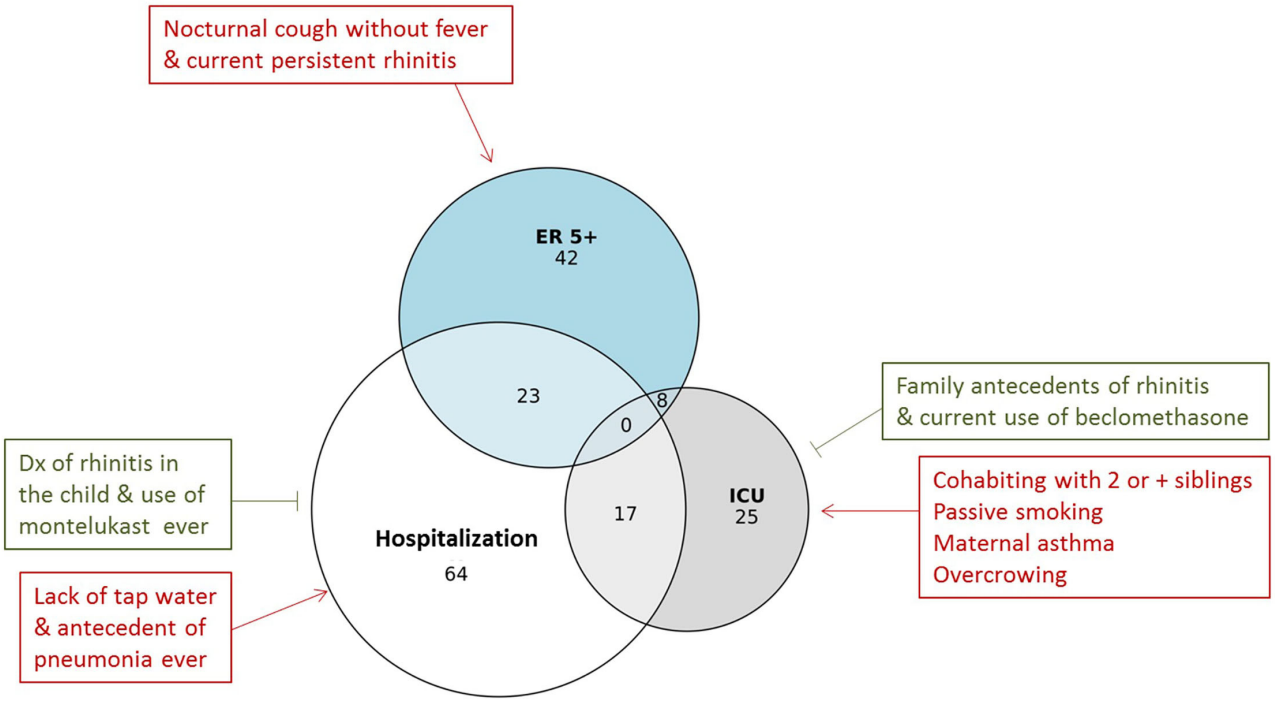

FIGURE 5 | Euler diagram for the overlap among children having had more than 5 ER visits due to wheezing (ER 5+) with those that had been hospitalized and/or admitted to the ICU due to wheezing ($n = 127$). Number of subjects is indicated in the circles. The risk factors and exposures associated to health care utilization are presented within boxes. Red indicates increased risk and green decreased risk.

ICU admission. These data support previous observations in a prospective observational study in this population showing that maternal asthma was the most significant factor associated with recurrent wheezing (24). The history of parasite expulsion was associated with increased risk of ICU admission but with marginal significance. This factor could serve as a proxy of the poor living conditions of these children but also could be related with the fact that migration of roundworms can induce pulmonary inflammation (41, 42). Since children with a history of ICU admission were less prone to be analyzed through SPT (44 vs. 69.6% without ICU admission) or serology (32 vs. 64.7% without ICU admission), we could not retrieve a large enough sample size to rule out or confirm involvement of atopy in ICU admission.

Previous studies have found associations between atopy indicators to be important risk factors for predicting asthma at school age (43). However, our results suggest that these factors may not be related to health care utilization for wheezing in this population. For instance, atopy as determined by serology was not associated with any outcome. Indeed, the proportion of preschool children with a positive SPT was low, including allergen sources such as milk and eggs (**Figure 2**). Further studies are needed to elucidate the biological processes implicated in the differences in prevalence of IgE sensitization when assessed by SPT and serology since this dissociation has been reported (44). A limitation of this study is that we did not evaluate other atopic biomarkers such as eosinophilia. Moreover, we did not analyze the presence of parasitic infection, which could also confound the IgE measurements against HDM extracts due to cross-reactivity (45, 46). Nevertheless, our observations indicate that IgE sensitization to HDM or total IgE was not associated with health care utilization during the wheezing episodes in this age group.

Recall bias is a potential source of confounding in this study, since the only wheezing episode documented by a physician was that by the medical staff at the ER visit. All the other ones were based on parental description. Since previous studies have shown up to 50% discordance between parental-described "wheeze" and physician-documented wheeze (47), here we documented the number of times the children were admitted to the ER due to wheezing, as well as hospitalizations, which are events with a reduced chance of bias due to incorrect parent-defined wheezing. Another limitation of this study is the lack of serology for common viruses or detection of virus in nasal or pharyngeal swabs. This is important for determining if the exacerbations are related to viral wheeze and lower respiratory tract infections (48). Further studies with a larger sample size, new statistical models (49), and appropriate procedures for random sampling and viral detection should be conducted to better define the wheeze phenotypes of these children with recurrent ER visits and those that are more likely to require hospitalizations and/or ICU admissions.

In conclusion, nocturnal cough without fever was the most significant factor associated with increased risk of

five or more ER visits during the previous year due to wheezing. On the other hand, children that require ICU admission due to wheezing reported several factors associated with poverty such as cohabiting with two or more siblings, overcrowding, passive exposure to smoke and lack of sewage facilities. Allergic sensitization was not associated with health care utilization although maternal asthma was associated with ICU admission. Moreover, poverty indicators should be considered as aggravating factors for wheezing and could be useful in the development of scores to grade and improve the approach and management of preschool recurrent wheezers from underdeveloped regions in the tropics.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of the Hospital Infantil Napoleon Franco Pareja (Act. 8-16/03/8), the parents provided written informed consent to participate for all the children and patient anonymity was preserved. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CM and JE-A: conception and design of the study. CM, LG, RR, and JE-A: acquisition of data. CM, NA, and JE-A: analysis and interpretation of data. CM and NA: drafting the article. CM, NA, LG, M-IE, and JE-A: revising it critically for important intellectual content. All authors approved the final version to be submitted.

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Pattern of Aeroallergen Sensitization and Quality of Life in Adult Thai Patients With Allergic Rhinitis

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The prevalence of allergic rhinitis (AR) is steadily rising in the Thai population, causing a major impact on the quality of life (QoL). Enhancing knowledge on common aeroallergens in the local setting helps in the appropriate prevention and management of AR. In this study, the demographic characteristics, clinical data, aeroallergen sensitization pattern, allergic symptoms, visual analog scale (VAS) score, and QoL are described. We evaluated the association between VAS, QoL, and severity of symptoms, except the aeroallergen sensitization pattern. We retrospectively reviewed the medical records of adult AR patients with a positive skin prick test (SPT) for at least one aeroallergen from January 2018 to May 2020. Standard descriptive and inferential statistics were used for analysis. A total of 366 patients were enrolled. Indoor aeroallergen sensitization and outdoor aeroallergen sensitization were observed in 32% and 7.9% of patients, respectively. Mono-sensitization was noted in 16.9% of patients, while poly-sensitization was noted in 83.1% of patients. Mites (65%) and sedge (39.3%) were the most common indoor and outdoor allergens. Nasal obstruction (74.6%), runny nose (63.7%), and nasal itchiness (61.5%) were the primary symptoms affecting the QoL. The association between VAS and symptom scores showed a trend of association with AR severity (Allergic Rhinitis and its Impact on Asthma [ARIA] classification) and VAS. AR has a significant effect on QoL in all domains of the validated generic (short-form-36, SF-36) and specific (rhino-conjunctivitis QoL questionnaire, Rcq-36) questionnaires. Mite and sedge remain the most common indoor and outdoor aeroallergens. The pattern of sensitization and number of aeroallergens were not associated with AR based on the ARIA guidelines. Meanwhile, symptoms of patients affected the QoL and VAS scores, which can be used as a quick and reliable tool for monitoring and stepping up or stepping down the treatment according to the next-generation guidelines. AR has a significant impact on the QoL of adult Thai patients.

Keywords: allergic rhinitis, skin prick test, aeroallergen, quality of life, visual analog scale

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INTRODUCTION

Allergic rhinitis (AR) is an immunoglobulin (Ig) E-mediated inflammation of the nasal mucosa induced after allergen exposure and has three cardinal symptoms: sneezing, nasal obstruction, and rhinorrhoea (1). AR may also be frequently associated with asthma, as emphasized by the Allergic Rhinitis and its Impact on Asthma (ARIA) document (2). The skin prick test (SPT) is considered a standard diagnostic method because of its accuracy, reproducibility, and affordability; internationally, it remains the most acceptable and cost-effective means of diagnosing AR (3, 4). The effective management of AR requires a precise diagnosis, which includes the identification of IgE-mediated inflammation (4). The optimal management includes adequate control of symptoms through the provision of patient education, environmental control, and the use of pharmaceutical therapies and immunotherapy (5).

Allergic rhinitis represents a global health problem, affecting 10–20% of the population. The prevalence of AR is steadily rising in Thailand, with no signs of abating. According to a previous study, the prevalence of AR increased from 37.9 to 50.6% (6). AR has a major impact on the physical, mental, and social functioning of Thai patients. AR impairs work, sleep, and emotional health. With its increasing incidence and the inadequate control of symptoms, AR causes a socioeconomic burden (6). The pattern of aeroallergen sensitization varies according to the geographical region due to differences in climate, urbanization, and lifestyle. However, the sensitization pattern constantly changes with the changes in economic conditions, level of industrialization, and lifestyle, due to the alterations in the prevailing circulating aeroallergens. Knowledge of the up-to-date data regarding the offending aeroallergen in a local setting at a particular time is important for the effective management of AR (3, 7).

According to recently published 19-year (1998–2017) data from an ENT allergy clinic, Faculty of Medicine Siriraj Hospital, the mite *Dermatophagoides pteronyssinus* (*Dp*) remains the most common indoor aeroallergen, while sedge remains the most common outdoor aeroallergen among the Thai population (4). This study aimed to analyse the pattern of aeroallergen sensitization and describe the demographic and clinical data related to AR. It also aimed to describe the quality of life (QoL) and determine the association between QoL and the severity of AR symptoms. The short-form-36 (SF-36) and rhino-conjunctivitis QoL questionnaire (Rcq-36) questionnaires are generic and disease-specific QoL questionnaires, which have been translated and validated to be used in the Thai population (8, 9). Thus, the present study assessed the impact of AR in Thai patients using the SF-36 and Rcq-36 questionnaires.

MATERIALS AND METHODS

This study was a retrospective chart review of adult patients diagnosed with AR by a positive SPT for at least one aeroallergen, conducted at the Rhinology and Allergy Unit, Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, between January 2018 and May 2020. Patients who were unable to completely fill out the case record forms or QoL questionnaires were excluded from our analysis. The following demographic and clinical data were obtained: age, gender, presenting symptoms, duration of symptoms, SPT results for various aeroallergens, the score of each domain in SF-36 (generic QoL questionnaire), the score of each domain in the Rcq-36 (specific questionnaire), and classification and severity of AR based on the ARIA guidelines. The patients were asked to discontinue taking oral antihistamines for 7 days prior to the test day. The visual analog scale (VAS) was used to determine the severity of AR symptoms (0–10 cm), and a diary card was provided to record the patient's symptoms for the last 7 days prior to the SPT.

The SPT was performed on the ventral aspect of the forearm by placing one drop of each allergenic extract 3-cm apart; then, the skin was pricked with a 26-gauge separate disposable needle in the middle of each allergen drop, using light pressure. The result was considered positive if the wheel diameter was larger than 3 mm and had an accompanying flare. Siriraj Allergen Vaccine (SAV), which contains standardized allergen extracts and has proven allergenic potency, was used (4, 10). Allergen extracts from mite *Dp*, American cockroach (*Periplaneta americana*), cats, dogs, molds (*Aspergillus* spp., *Penicillium* spp., and

TABLE 1 | Demographic and clinical characteristics of patients ($n = 366$).

Characteristic	Value
Sex [*n* (%)]	
Female	236 (64.5%)
Male	130 (35.5%)
Age (mean ± SD)	
Female	35.7 ± 12.4
Male	33.2 ± 12.6
Duration of symptom(year) (mean + SD)	
Female	9.7 ± 10.1
Male	9.0 ± 7.8
Age of onset (year) (mean ± SD)	
Female	25.5 ± 13.6
Male	23.3 ± 13.4
Type of aeroallergen [*n* (%)]	
Indoor	117 (32%)
Outdoor	29 (7.9%)
Both	220 (60.1%)
Type of sensitization [*n* (%)]	
Mono-sensitization (one allergen)	62 (16.9%)
Poly-sensitization (more than one allergen)	304 (83.1%)
Symptoms [*n* (%)]	
Nasal obstruction	273 (74.6%)
Runny nose	233 (63.7%)
Nasal itchiness	225 (61.5%)
Sneezing	185 (50.5%)
Itchy eyes	183 (50%)
Cough	95 (26%)
Excessive tearing	78 (21.3%)

TABLE 2 | Type of aeroallergen, type of sensitization, symptom score, and VAS score classified based on the ARIA guideline ($n = 366$).

	Mild intermittent ($n = 51$)	Moderate to severe intermittent ($n = 82$)	Mild persistent ($n = 35$)	Moderate to severe persistent ($n = 198$)	*p*-value
Type of aeroallergen [*n* (%)]					
Indoor	19 (16.2)	20 (17.1)	10 (8.5)	68 (58.1)	*0.54*
Outdoor	5 (17.2)	5 (17.2)	2 (6.9)	17 (58.6)	
Both	27 (12.3)	57 (25.9)	23 (10.5)	113 (51.4)	
Type of sensitization [*n* (%)]					
Mono-sensitization	10 (16.1)	11 (17.7)	4 (6.5)	37 (59.7)	*0.49*
Poly-sensitization	41 (13.5)	71 (23.4)	31 (10.2)	161 (53.0)	
Symptom score (0–21) (mean ± SD)					
Nasal itchiness	2.2 ± 3.0	3.0 ±4.0	3.9 ± 5.3	6.0 ± 5.8	*<0.001*
Sneezing	2.7 ± 3.0	3.7 ±3.5	5.0 ± 5.4	7.0 ± 5.7	*<0.001*
Nasal obstruction	2.9 ± 3.5	4.9 ±5.1	7.4 ± 6.2	10.0 ± 6.3	*<0.001*
Runny nose	2.0 ±2.7	3.8 ± 4.0	4.2 ± 4.5	6.6 ± 5.9	*<0.001*
Post-nasal drip	1.8 ± 3.5	3.1 ± 4.6	5.2 ± 6.4	5.5 ± 5.9	*<0.001*
Smell loss	2.0 ±4.4	2.1 ± 4.4	2.7 ± 5.0	3.0 ± 5.5	*0.003*
Itchy eyes	1.3 ±2.6	3.4 ± 4.9	3.9 ± 5.4	5.3 ± 6.1	*0.002*
VAS (0–10) (mean ± SD)	2.3 ± 1.4	4.8 ± 2.3	3.4 ± 2.3	6.1 ± 2.0	*<0.001*

$p < 0.05$: Significant difference between the type of aeroallergen and type of sensitization with ARIA classification using the chi-square test.
$p < 0.05$: Significant difference between symptom scores and VAS with ARIA classification using the independent-samples Kruskal-Wallis Test.
VAS, visual analog scale; ARAI, Allergic Rhinitis and its Impact on Asthma.

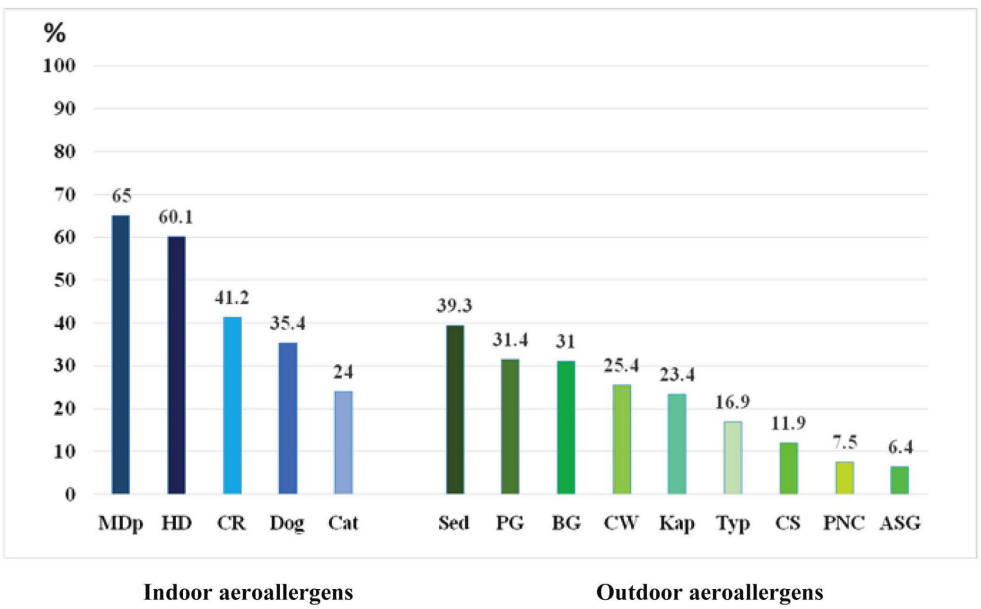


FIGURE 1 | Pattern of aeroallergen sensitization ($n = 366$). Abbreviations: MDp, mite/*Dermatophagoides pteronyssinus*; HD, house dust; CR, cockroach; Sed, sedge; PG, para grass; BG, bermuda grass; CW, careless weed; Kap, Kapok; Typ, *Typha*; CS, *Cladosporium*; PNC, *Penicillium*; ASG, *Aspergillum*.

Cladosporium spp.), Bermuda grass (*Cynodon dactylon*), para grass (*Brachiaria mutica*), sedge (*Cyperaceae*), careless weed (*Amaranthus palmeri*), and Kapok (*Ceiba pentandra*) were used in our center. Histamine and normal saline were used as positive and negative controls, respectively (4). All SPTs were performed by qualified technicians.

Validated Thai versions of the SF-36 (8) and Rcq-36 questionnaires (9, 11) were used in this study. The AR patients in our center were instructed to draw a cross on the horizontal line of the VAS at the specific point that most accurately indicated their symptom severity (from 0 to 10 cm). The symptom diary card is a form that contains a list of AR-related symptoms.

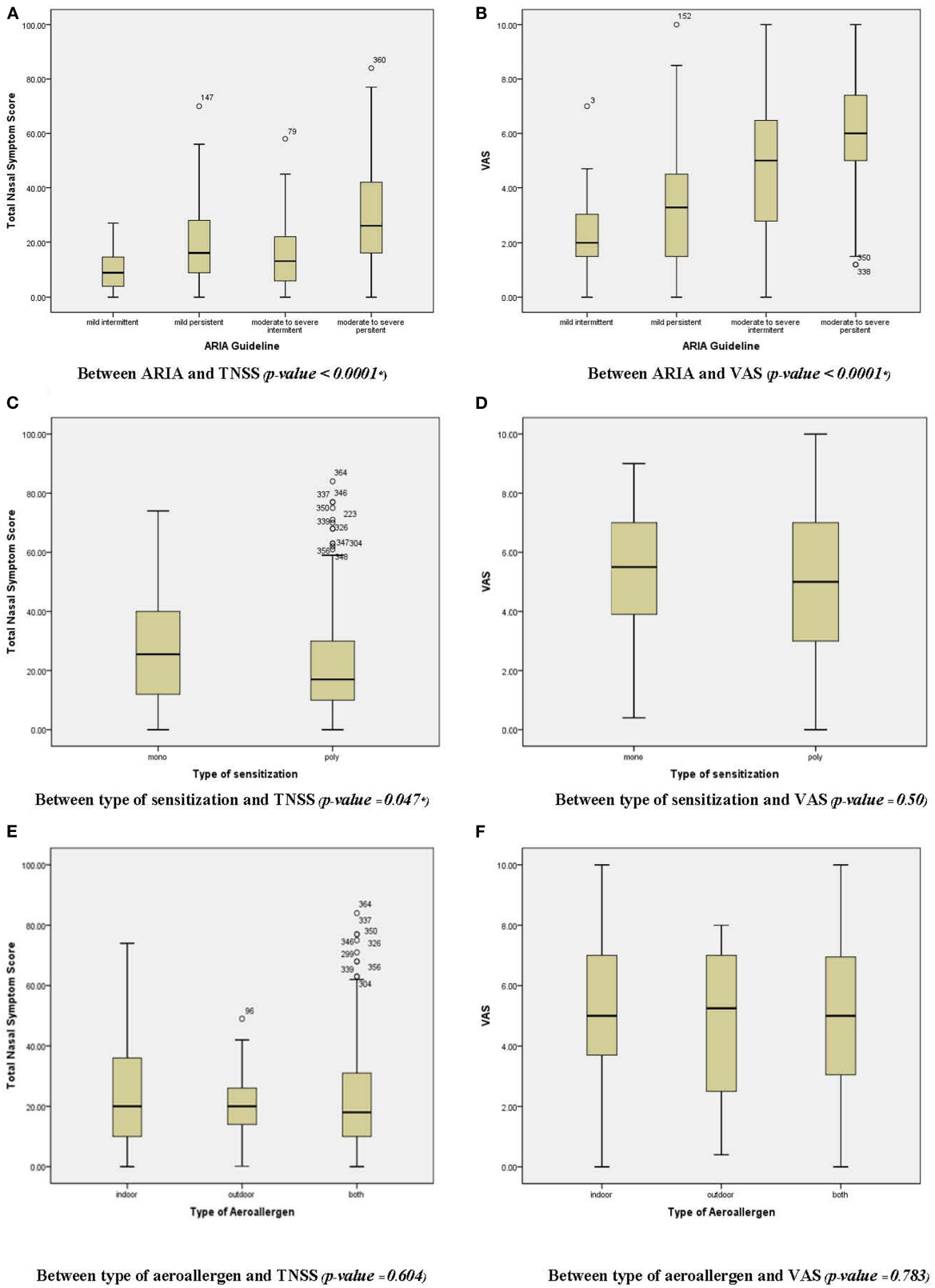


FIGURE 2 | Association among TNSS, VAS score, ARIA classification, and type of sensitization and aeroallergen. TNSS, the total nasal symptom score; VAS, visual analog scale; ARAI, Allergic Rhinitis and its Impact on Asthma. *$p < 0.05$.

TABLE 3 | Type of aeroallergen and each domain of the SF-36 and Rcq-36 questionnaires ($n = 366$).

Domains	Type of aeroallergen		*p-value*
	Mono-sensitization ($n = 62$)	Poly-sensitization ($n = 304$)	
SF-36			
Physical functioning (PF)	77.02 ± 20.37	80.48 ± 20.31	*0.23*
Role physical (RP)	68.15 ± 41.03	75.58 ± 35.32	*0.14*
Bodily pain (BP)	66.18 ± 21.33	70.12 ± 19.42	*0.18*
General health (GH)	42.34 ± 19.98	44.64 ± 20.07	*0.41*
Vitality (VT)	52.02 ± 15.46	55.26 ± 17.96	*0.15*
Social functioning (SF)	71.77 ± 23.51	75.41 ± 21.99	*0.27*
Role emotional (RE)	65.05 ± 43.70	69.96 ± 38.82	*0.38*
Mental health (MH)	62.65 ± 16.58	66.86 ± 16.81	*0.72*
Rcq-36			
Rhinitis symptoms (RS)	62.80 ± 22.25	62.64 ± 22.85	*0.96*
Eye symptoms (ES)	74.80 ± 19.86	76.21 ± 21.22	*0.63*
Other symptoms (OS)	71.33 ± 21.13	73.01 ± 19.90	*0.55*
Role limitation (RL)	84.01 ± 16.82	81.09 ± 22.17	*0.24*
Physical functioning (PF)	83.47 ± 19.29	83.58 ± 20.66	*0.97*
Sleep (sleep)	70.43 ± 31.02	71.13 ± 29.04	*0.86*
Social functioning (SF)	79.70 ± 24.87	80.04 ± 25.74	*0.92*
Emotions (E)	64.84 ± 24.58	67.20 ± 26.90	*0.52*

$p < 0.05$: Significant difference between the type of aeroallergen and each domain of the SF-36 and Rcq-36 questionnaires using unpaired t-test. Req, rhino-conjunctivitis QoL, questionnaire.

TABLE 4 | Type of sensitization and each domain of the SF-36 and Rcq-36 questionnaires ($n = 366$).

Domains	Type of sensitization			*p-value*
	Indoor aeroallergen ($n = 117$)	Outdoor aeroallergen ($n = 29$)	Both groups ($n = 220$)	
SF-36				
Physical functioning (PF)	79.40 ± 20.81	84.83 ± 12.78	79.50 ± 20.86	*0.22*
Role physical (RP)	73.50 ± 36.74	81.90 ± 33.34	73.75 ± 36.64	*0.14*
Bodily pain (BP)	67.75 ± 21.44	72.38 ± 18.33	69.97 ± 19.04	*0.15*
General health (GH)	44.79 ± 19.74	43.55 ± 19.15	44.06 ± 20.40	*0.41*
Vitality (VT)	54.83 ± 17.47	56.03 ± 15.38	54.48 ± 17.98	*0.19*
Social functioning (SF)	75.00 ± 23.56	73.28 ± 20.52	74.89 ± 21.86	*0.24*
Role emotional (RE)	68.38 ± 39.60	71.26 ± 41.52	69.24 ± 39.64	*0.38*
Mental health (MH)	65.09 ± 18.39	65.10 ± 16.17	66.84 ± 16.06	*0.07*
Rcq-36				
Rhinitis symptoms (RS)	62.23 ± 21.98	67.46 ± 18.70	62.27 ± 23.59	*0.96*
Eye symptoms (ES)	76.07 ± 19.92	80.17 ± 17.36	75.36 ± 21.95	*0.63*
Other symptoms (OS)	74.62 ± 19.86	74.81 ± 18.57	71.44 ± 20.39	*0.55*
Role limitation (RL)	82.76 ± 18.56	85.63 ± 15.41	80.42 ± 23.31	*0.33*
Physical functioning (PF)	83.90 ± 19.78	84.20 ± 20.45	83.30 ± 20.82	*0.97*
Sleep (sleep)	72.01 ± 29.57	72.13 ± 29.65	70.34 ± 29.29	*0.86*
Social functioning (SF)	79.06 ± 26.34	85.92 ± 18.24	79.70 ± 25.95	*0.92*
Emotions (E)	67.18 ± 26.05	71.90 ±22.69	65.93 ± 27.22	*0.52*

$p < 0.05$: Significant difference between the type of sensitization and each domain of the SF-36 and Rcq-36 questionnaires using analysis of variance. Req, rhino-conjunctivitis; QoL, questionnaire.

Patients were asked to recollect the severity of the given symptom and provide a score accordingly. The list of symptoms provided by the study patients included an itchy nose, sneezing, nasal obstruction, runny nose, post-nasal drip, loss of smell, itchy eyes, and others. Each symptom was scored from 0 to 3: 0 = none, 1 = mild, 2 = moderate, or 3 = severe. The total nasal symptom score (TNSS) is the sum of scores for nasal congestion, sneezing, nasal itching, and rhinorrhoea at each time point. TNSS was calculated by adding the score for each symptom, with a total score of 12 points (12).

Data processing and analysis were performed using PASW Statistics (SPSS Inc., Chicago, IL, USA). The data were expressed as frequency and percentage for categorical data or mean ± SD for continuous data. Normally distributed continuous data were evaluated using the one-sample Kolmogorov-Smirnov test. The data were classified according to the ARIA guidelines. The differences in the type of aeroallergen and type of sensitization among the AR severity groups (ARIA classification) were determined by chi-square tests. The association between TNSS, VAS, and QoL scores in the SF-36 and Rcq-36 questionnaires and type of aeroallergen, pattern of sensitization, or ARIA classification was analyzed using the Kruskal-Wallis test; meanwhile, the association of TNSS, VAS, and pattern of sensitization was determined using the Mann-Whitney *U*-test for non-normally distributed data and using unpaired *t*-tests and ANOVA for normally distributed data. Clinical symptoms and the pattern of sensitization were evaluated for association with severity and QoL. The factors associated with the pattern of sensitization and ARIA classification with a p-value <0.05 using univariate analysis by stepwise method and multivariate analysis are shown as *p*-value and odds ratio (OR) with 95% CI. A *p* value < 0.05 was considered significant.

This study was approved by the Siriraj Hospital Institutional Review Board (approval number: **Si 296/2019**).

RESULTS

The patient demographic and clinical characteristics are shown in **Table 1**. A total of 366 patients who fulfilled the inclusion criteria were included in our study. Among them, 236 (64.5%) were women and 130 (34.5%) were men. The mean age of the female participants was 35.7 ± 12.4 years, while that of the male participants was 33.2 ± 12.6 years. The mean duration of symptoms was 9.7 ± 10.1 years in women and 9.0 ± 7.8 years in men. The mean ages of onset of AR were 25.5 ± 13.6 years in women and 23.3 ± 13.4 years in men.

Poly-sensitization was observed in 304 (83.1%) patients, while mono-sensitization was observed in 62 (16.9%) patients. Sensitization to indoor aeroallergens was noted in 117 (32%) patients, while sensitization to outdoor allergens was noted in 29 (7.9%) patients. Sensitization to both the aeroallergens was observed in 220 (60.1%) patients. Nasal obstruction (74.6%),

TABLE 5 | Factors associated with the pattern of sensitization and ARIA classification.

Factor	Univariate analysis		
	Type of sensitization	Type of aeroallergen	ARIA classification
	p-value	*p-value*	*p-value*
Age	*0.77*	*0.66*	*0.88*
Duration of symptom	*0.94*	*0.04**	*0.88*
Age of onset	*0.76*	*0.04**	*0.66*
Nasal itchyness	*0.05**	*0.34*	*<0.001***
Sneezing	*0.69*	*0.24*	*<0.001***
Obstruction	*0.1*	*0.88*	*<0.001***
Runny nose	*0.83*	*0.07*	*<0.001***
Itchy eyes	*0.11*	*0.41*	*<0.001***
TNSS	*0.15*	*0.18*	*<0.001***
VAS	*0.27*	*0.47*	*<0.001***
SF-36			
Physical functioning (PF)	*0.13*	*0.18*	*<0.001***
Role physical (RP)	*0.29*	*0.4*	*<0.001***
Bodily pain (BP)	*0.06*	*0.15*	*<0.001***
General Health (GH)	*0.73*	*0.96*	*<0.001***
Vitality (VT)	*0.18*	*0.7*	*<0.001***
Social functioning (SF)	*0.19*	*0.98*	*<0.001***
Role emotional (RE)	*0.2*	*0.66*	*0.01**
Mental health (MH)	*0.03**	*0.48*	*<0.001***
Rcq-36			
Rhinitis ymptoms (RS)	*0.63*	*0.72*	*<0.001***
Eye symptoms (ES)	*0.22*	*0.23*	*<0.001***
Other symptoms (OS)	*0.21*	*0.27*	*<0.001***
Role limitation (RL)	*0.81*	*0.24*	*<0.001***
Physical functioning (PF)	*0.68*	*0.85*	*<0.001***
Sleep (sleep)	*0.87*	*0.87*	*<0.001***
Social functioning (SF)	*0.85*	*0.43*	*<0.001***
Emotions (E)	*0.19*	*0.22*	*<0.001***

** and ** indicates significant differences between the pattern of sensitization including type of sensitization, type of aeroallergen and ARIA classification and factors. *indicates $p < 0.05$ and ** indicates $p < 0.001$. VAS, visual analog scale; ARAI, Allergic Rhinitis and its Impact on Asthma; TNSS, the total nasal symptom score.*

runny nose (63.7%), and nasal itchiness (61.5%) were the most common symptoms observed in AR patients in this study. These symptoms were frequently observed in patients with moderate-to-severe intermittent and persistent AR. Based on the ARIA classification, a majority of the patients had moderate-to-severe persistent AR (54%), followed by moderate-to-severe intermittent AR (22%), mild intermittent AR (14%), and mild persistent AR (10%) (**Table 2**).

The frequency of sensitization to various aeroallergens, from January 2018 to May 2020, is shown in **Figure 1**. The indoor aeroallergen was most frequently sensitized against the mite *Dp* (65%) followed by house dust (60.1%), cockroach (41.2%), dog (35.4%), and cat (24%). Meanwhile, the outdoor aeroallergen was most frequently sensitized against sedge (39.3%) followed by para grass (31.4%), Bermuda grass (31%), careless weed (25.4%), Kapok (23.4%), *Typha* (16.9%), *Cladosporium* (11.1%), *Penicillium* (7.5%), and *Aspergillus* (6.4%) (**Table 2**).

Regarding the determinants of AR severity, a chi-square test revealed no significant relationship between the type of aeroallergen ($p = 0.54$) and the type of sensitization ($p = 0.49$) (**Table 2**). However, a significant difference was observed between the symptom score, VAS score, and ARIA classification using the independent-sample Kruskal-Wallis test ($p < 0.05$). TNSS is significantly associated with the type of sensitization (**Figure 2**). An increased VAS score was associated with symptom severity and ARIA classification. In contrast, no significant association was found between the type of aeroallergen, sensitization, and the SF-36 and Rcq-36 domains (**Tables 3**, **4**). Factors associated with the pattern of sensitization and ARIA classification are shown in **Table 5**. There was no association between the pattern of sensitization and patient's characteristics and symptoms. However, there was association between ARIA classification, symptom severity (TNSS and VAS), and QoL (**Table 6**). **Figures 3**, **4** show a comparison of the types of aeroallergens and sensitization among the SF-36 and Rcq-36 domains. Furthermore, when each domain of the generic SF-36 questionnaire was compared with the ARIA classification using ANOVA, a significant difference was observed ($p < 0.05$). The QoL in all domains was poorer as the severity of AR increases. Bodily pain, general health, and vitality were the most severely affected domains. The disease-specific QoL questionnaire (Rcq-36) also yielded poorer QoL in all domains, especially for rhinitis symptoms (54.5%), sleep (65.5%), and emotions (57.5%), with ANOVA yielding significant results ($p < 0.05$; **Figure 5**).

DISCUSSION

The Mite was the most common sensitizing indoor aeroallergen (65%), while sedge remained the most common sensitizing outdoor aeroallergen (39.3%) reported in our study, which was conducted from January 2018 to May 2020. When compared to the 19-year (1998–2017) data from the same center (4), no significant changes in the distribution of sensitization patterns were observed. However, the percentage of sensitization against mites increased from 54.8 to 65%, while that of sensitization against cockroaches increased from 36 to 41.2%. There was no significant change in the percentage sensitization pattern in the outdoor aeroallergen group. Sedge, para grass, and Bermuda grass were the top three outdoor aeroallergens that caused sensitization in our study; this finding is similar to those of a previous study conducted in the same center. This result indicates that both indoor and outdoor allergens remain the cause of AR among Thai patients. A study from another tertiary care hospital in Bangkok reported a mite *Dp* sensitization rate of 50.1% within a 12-year period (from January 2004 to December 2015) (13), which establishes the fact that there is an upwards trend in mite sensitization in adult Thai AR patients.

House dust mite is the most common sensitizing aeroallergen worldwide and causes sensitization in up to 90% of Asian atopic patients (7, 14). The mite sensitization rates were 57.5% in South

TABLE 6 | Independent risk ration of univariable and multivariable analysis by multinomial logistic regression.

	ARIA Classification					
	Moderate to severe intermittent		Mild persistent		Moderate to severe persistent	
Univariable analysis	**Crude Odds ratio (95% CI)**	***p-value***	**Crude Odds ratio (95% CI)**	***p-value***	**Crude Odds ratio (95% CI)**	***p-value***
Itchy eye	1.16 (1.04, 1.29)	*0.01**	1.18 (1.045, 1.33)	*0.007**	1.23 (1.11, 1.37)	*<0.0001***
TNSS	1.06 (1.02, 1.11)	*0.002**	1.10 (1.05, 1.15)	*<0.0001***	1.14 (1.09, 1.18)	*<0.0001***
VAS	2.02 (1.59, 2.58)	*<0.0001***	1.45 (1.11, 1.90)	*<0.0001***	2.74 (2.14, 3.50)	*<0.0001***
Multivariable analysis (Enter method)	**Adjusted Odds ratio (95% CI)**	***p-value***	**Adjusted Odds ratio (95% CI)**	***p-value***	**Adjusted Odds ratio (95% CI)**	***p-value***
Itchy eye	1.02 (0.90, 1.16)	*<0.0001***	1.06 (0.93, 1.21)	*0.401*	1.00 (0.89, 1.13)	*0.977*
TNSS	1.01 (0.96, 1.06)	*0.8849*	1.06 (1.00, 1.11)	*0.045**	1.08 (1.03, 1.13)	*0.002**
VAS	1.20 (1.54, 2.59)	*<0.0001***	1.24 (0.93, 1.67)	*0.159*	2.35 (1.81, 3.05)	*<0.0001***

** and ** indicates significant differences ARIA classification and itchy eye, TNSS and VAS. * indicates p-value was <0.05 and ** indicates p-value was <0.001.*

coast China (15), 97.4% in the Philippines (7), 68.5% in Singapore (16), 21.4% in South Korea (17), and 63% in Hong Kong (3). The aeroallergen sensitization pattern in Thai adults is consistent with the findings from Asian countries with the similar climatic conditions. The hot and humid and tropical climate of Thailand, throughout the year, is favorable for the mite, allowing it to thrive.

The worsening air pollution in Bangkok and adjacent areas over the years may have impacts on allergens, along with changes in atmospheric variables, such as CO_2 concentration, temperature, rainfall, humidity, and wind speed and direction (18, 19). A previous study in Thailand reported that pollen is present in the air throughout the year, and grass and weed pollen, e.g., Bermuda grass (*Cynodon dactylon*), para grass (*Panicum purpurascens*), sedge (*Carex species*), and careless weed (*Amaranthus hybridus*), is more commonly found in Bangkok than tree pollen (6). These findings might explain the upwards trend in mite sensitization and persistence in the sensitization pattern for grasses (Bermuda grass, sedge, and para grass) over the last two decades, despite the fact that Bangkok is an urban center and has relatively less greenery.

Lombardi et al. (20) reported that AR patients frequently self-managed with over-the-counter medications, with a physician-based diagnosis made in only 60% of patients. The next-generation ARIA care pathway stresses self-care and self-medication with certain over-the-counter drugs, enabling the physician to step-up or step-down the treatment based on the VAS score (21). Nasal obstruction (74.6%), runny nose (63.7%), and nasal itchiness (61.5%) were the three primary symptoms present in our AR patients. Based on our findings, the majority of these symptoms are present in patients with moderate-to-severe AR, irrespective of whether it is intermittent or persistent. These findings suggest that symptom severity matters more than the symptom longevity for moderate-to-severe disease and reiterates the importance of self-care and the self-medicate care pathway.

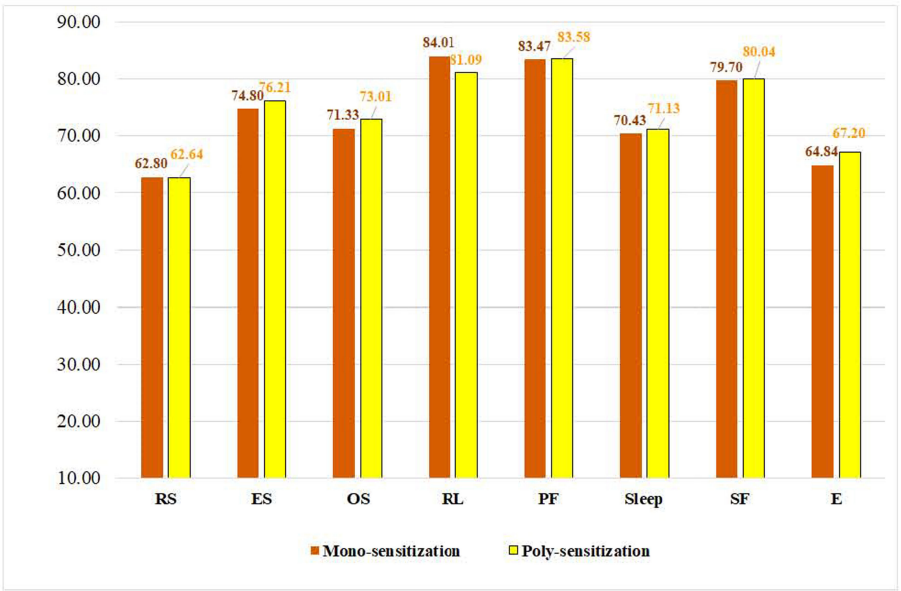
Supporting the concept of "one airway-one disease", 26% of AR patients in our study had cough, which re-emphasizes the fact that 40% of AR patients will also have concurrent bronchial asthma (22). In addition, 13.9% of moderate-to-severe persistent AR patients had cough, while only 6% of moderate-to-severe intermittent AR patients had cough.

Among the total number of patients recruited in our study ($n = 366$), 64.5% were women, while only 35.5% were men. However, no significant gender discrepancies were observed in terms of mean age, mean duration of symptoms, and mean age of onset of AR. The female predominance might be attributed to the better health awareness among women, as suggested in a previous study (4).

Visual analog scale is a simple and quantitative method that can be used for the quantitative evaluation of the severity of AR and for monitoring the efficacy of therapeutic interventions (23). The next-generation ARIA guideline uses an algorithm based on the VAS scores for the selection of pharmacotherapy for AR patients and to determine whether the treatment should be stepped up or stepped down depending on the status of disease control (24). In fact, the phase 3 ARIA initiative is based on Mobile Airways Sentinel Network, which aimed to initiate digitally enabled, integrated, person-centered care for rhinitis and asthma multi-morbidity using real-world evidence (25). The real-life-integrated care pathway has been adapted into the German healthcare system. An algorithm suitable for the German healthcare system using the VAS was devised and digitalised to step-up or step-down AR treatment (21). In Thailand, no previous study has reported the association between VAS scores and AR severity. Our study revealed a significant association between VAS scores and AR severity, which means that VAS can be used as a guide in the treatment and follow-up of our AR patients. The findings from our study are an important step toward the implementation of the real-world-integrated care pathway in the Thai population for the management of AR. However, in the present study, we did not assess the changes in VAS scores after initiation of treatment. Hence, further research is needed to support our findings.

A previous study in Thailand using the Thai version of the SF-36 questionnaire reported that AR patients had significantly

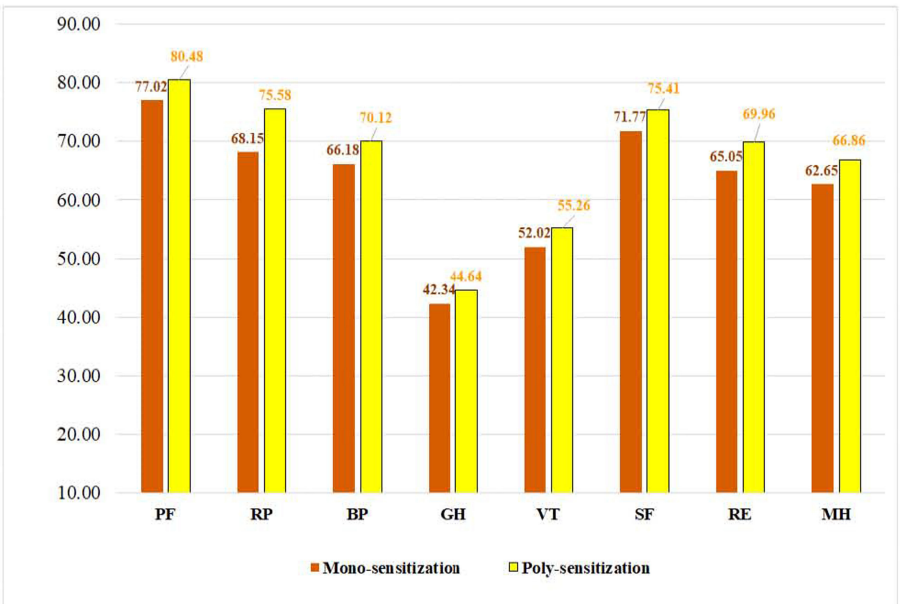
A



Comparison of each domain score of SF-36

PF = physical functioning | RP = pole physical | BP = bodily pain | GH = general health
VT = vitality | SF = social functioning | RE = role emotional | MH = mental health

B



Comparison of scores in each domain of the Rcq-36 questionnaire

RS = rhinitis symptoms | ES = eye symptoms | OS = other symptoms | RL = role limitation
PF = physical functioning | SP = sleep | SF = social functioning | E = emotion

FIGURE 3 | Comparison of QoL, SF-36, and Rcq-36 scores among types of aeroallergens. QoL, quality of life; SF, social functioning; Rcq, rhino-conjunctivitis QoL questionnaire; RS, rhinitis symptoms; ES, eye symptoms; OS, other symptoms; PF, physical functioning; SP, sleep; E, emotion. ($p < 0.05$: Significant difference between the type of aeroallergen and each domain of the SF-36 and Rcq-36 questionnaires using unpaired *t*-test).

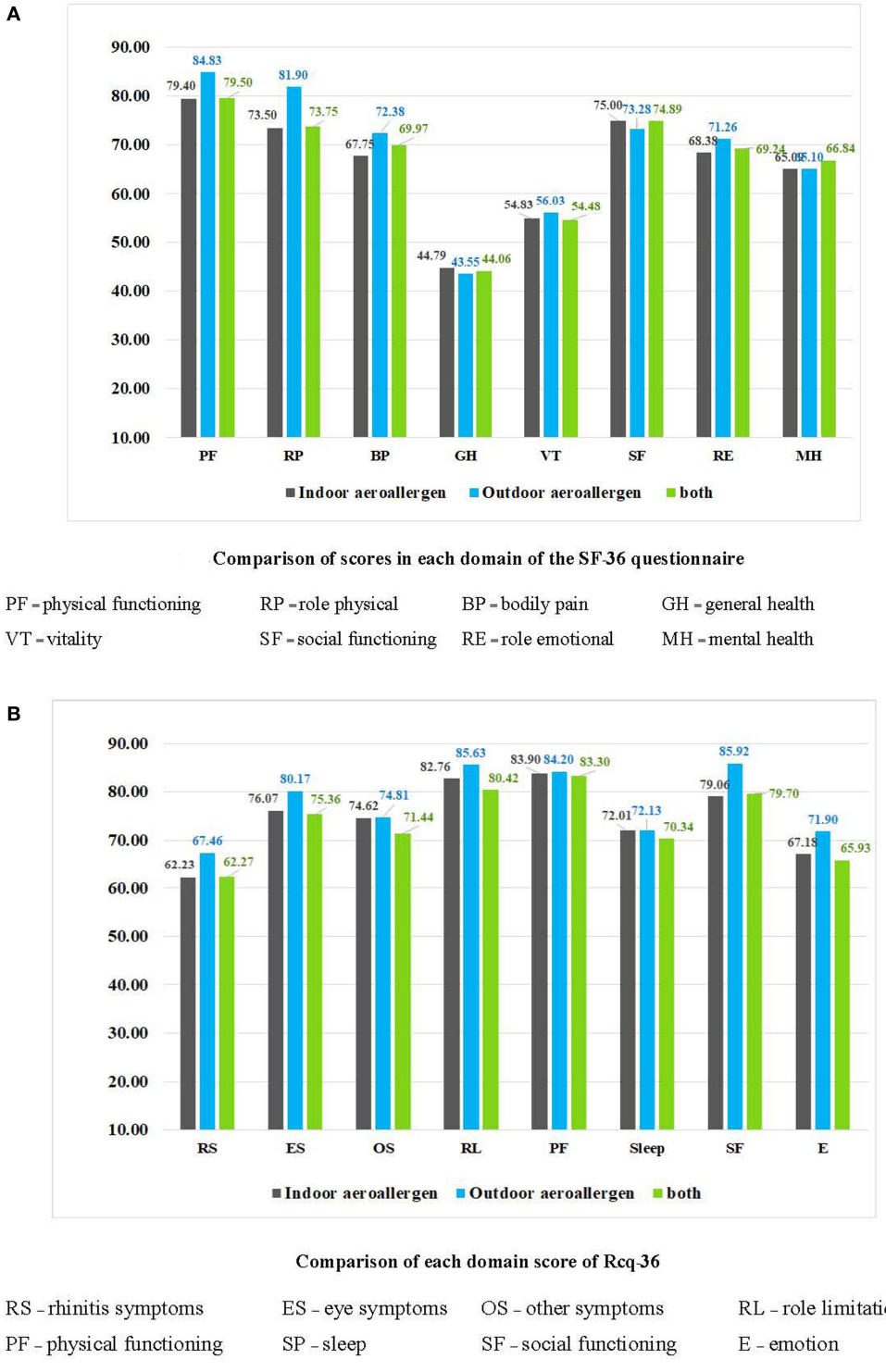


FIGURE 4 | Comparison of QoL, SF-36, and Rcq-36 scores among the different types of sensitizations. QoL, quality of life; SF, social functioning; Rcq, rhino-conjunctivitis. ($p < 0.05$: Significant difference between the type of sensitization and each domain of the SF-36 and Rcq-36 questionnaires using analysis of variance).

impaired QoL scores compared with healthy individuals in all aspects, except the social functioning dimension. The same study also reported that the Rcq-36 questionnaire showed a higher correlation with the symptom scores compared with the SF-36 questionnaire, additionally including information on sleep and productivity (6). In our study, the analysis of both SF-36 and

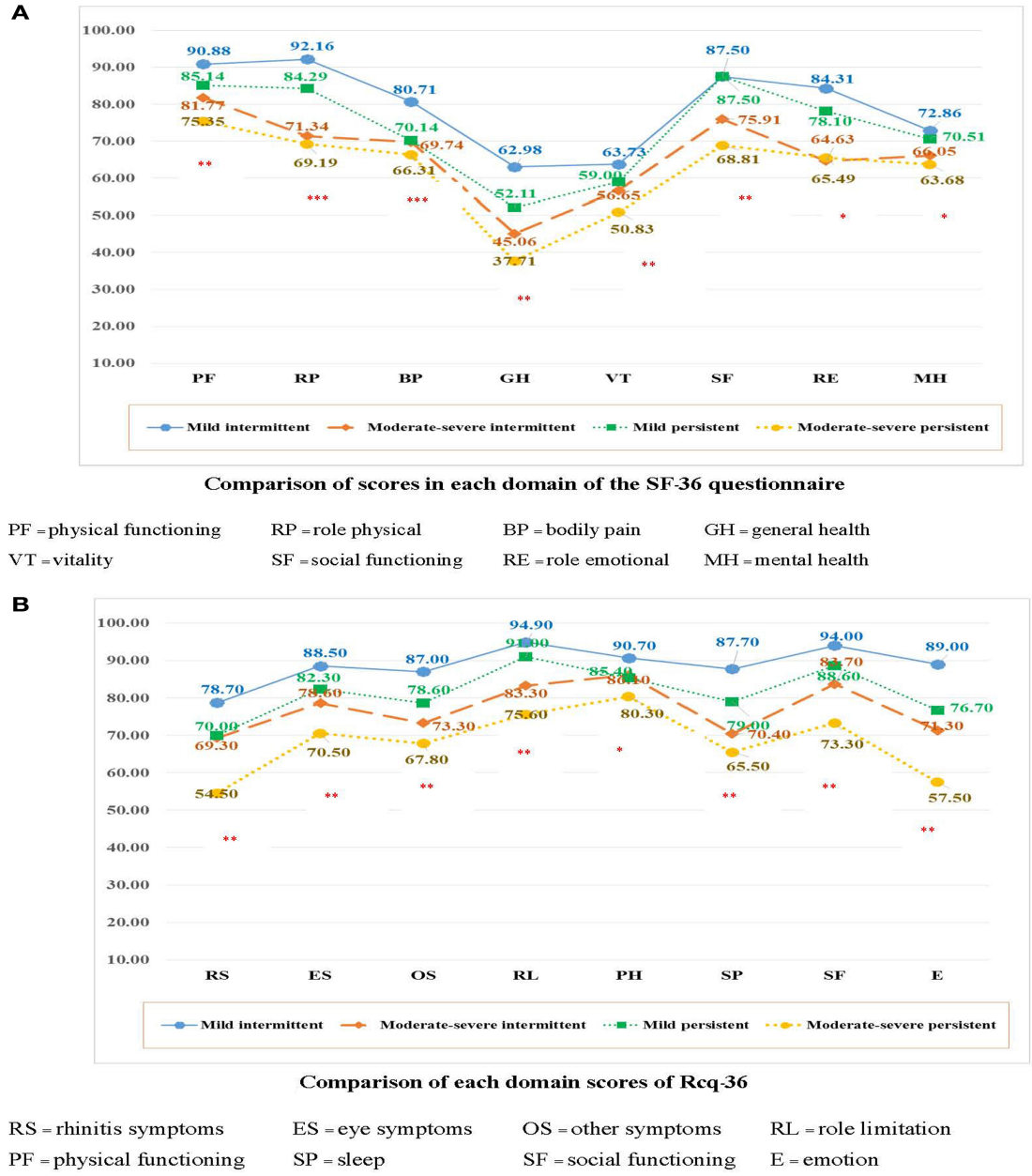


FIGURE 5 | Comparison of QoL between the ARIA classifications (36). QoL, quality of life; ARIA, Allergic Rhinitis and its Impact on Asthma. (*$p < 0.05$, **$p < 0.001$: Significant difference among the ARIA classifications using analysis of variance).

Rcq-36 questionnaires to assess the QoL showed the impact on all domains of health. The comparison of each domain of health with various categories of AR revealed that the QoL score became poorer as the severity of AR increased, which was significant ($p > 0.05$). Bodily pain, general health, and vitality were the most affected domains in the SF-36 questionnaire. Meanwhile, rhinitis symptoms, sleep, and emotions were the most affected domains in the analysis of the disease-specific questionnaire (Rcq-36). The findings from our study reaffirm that AR continues to be significantly affecting the QoL of the Thai population. Adapting and advocating the next-generation ARIA guideline with the use of real-world evidence and an integrated care pathway in the Thai population may fill the gap and adequately address the needs of AR patients.

Ciprandi and Cirillo (2) reported that the severity of symptoms was higher in poly-sensitized patients than in mono-sensitized patients. A previous study conducted in the Italian population suggested that mono-sensitization and poly-sensitization constitute two different phenotypes of AR. A similar study conducted in Malaysia demonstrated that sensitization

to two or more aeroallergens was significantly associated with moderate-to-severe persistent AR (26). Previous studies from Thailand reported that all of the clinical parameters significantly affected the QoL of patients (9, 22), but did not mention the effect of sensitization status. A recent study reported that sensitization status did not show a significant association with QoL (4), but this study did not mention the effect of the number of sensitization on disease severity or QoL. In our study, 83.1% of the AR patients exhibited poly-sensitization, while only 16.9% exhibited mono-sensitization. The chi-square test between ARIA classification of AR and number of sensitizations showed no significant association ($p = 0.49$). There may be several factors that can have an effect on the clinical severity of AR due to the number of sensitizing aeroallergens. This should be addressed in future research. Similarly, our study also revealed that the type of aeroallergen and number of sensitizations had no association with VAS score and TNSS.

Our study has some limitations. It was retrospective in nature and only used a 2-year dataset. Our findings would have been better supported if we had assessed the significance of the changes in VAS scores and QoL after the initiation of treatment. In addition, due to the prevailing coronavirus disease 2019 pandemic, only a few SPTs were performed from March to May 2020.

CONCLUSION

Mite and sedge remain the most common sensitizing indoor and outdoor aeroallergens in adult Thai patients with AR. The VAS scores are significantly associated with AR severity. VAS can be used as a quick and reliable tool in adult Thai patients with AR to monitor and step-up or step-down treatment. AR has a significant impact on the QoL of adult Thai patients, and the severity of AR is not associated with the number and type of aeroallergen.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Siriraj Institutional Review Board. Certificate of Approval No. Si 296/2019. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

PK: data collection, methodology, formal analysis, project administration, designed and wrote the first draft of the manuscript, and writing—review and editing. BP: data curation, statistical analysis, visualization, and writing—review and editing. KT: data collection and curation. PT: conceptualization, methodology, project administration, supervision, and editing. All authors contributed to the article and approved the submitted version.

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