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Reactivity Skin-Prick Test with Common Allergens and Their Genetic Association with Polymorphisms in *IL-9, IL-13, IL-4, IL-5 & IL-4Rα Genes* among Asthmatics in Sudan

Amel Osman Gundi[1, *], Fatima Omer Hamed[2], Maha Hassan Agraa[1], Hiba Salaheldin Mohamed[3]

[1]Department of Biochemistry, National Ribat University, Khartoum, Sudan

[2]Department of Public Health Protection, Dubai Health Authority, Dubai, United Arab Emirates (UEA)

[3]Department of Biology, Taibah University, Medina, Kingdom of Saudi Arabia (KSA)

Email address:

ameljundi@gmail.com (Amel Osman Gundi), agraa35@gmail.com (Maha Hassan Agraa), foahamed63@gmail.com (Fatima Omer Hamed), hmohamedahmed@taibahu.edu.sa (Hiba Salaheldin Mohamed)

[*]Corresponding author

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Abstract: Atopy may be defined as the production of abnormal amounts of Immunoglobulin E (IgE) antibodies in response to aeroallergens, assessed by positive skin-prick test (SPT) responses. Allergic asthma is the result of a complex multifactorial interplay between genes and the environment. The interaction of genetic and environmental factors may increase the complexity associated with allergic asthma. We investigated the degree of association of skin reactivity and their association with seven polymorphisms in *IL-4, IL-5, IL-13, and IL-9 genes* on chromosome 5q31-q33 region, and three polymorphisms in the *IL-4Rα gene* on chromosome 16 p contributing to allergic asthma in a population-based sample of Sudanese families. 70 families, including (150 cases and 140 controls), were recruited. Asthmatic status was confirmed by pulmonary function tests (PFTs); a SPT was done using 14 aeroallergens. Genotyping for 10 single-nucleotide polymorphisms (SNPs); one SNP in the *IL-4 gene;* one SNP in the *IL-5 gene*; two SNPs in the *IL-13 gene*; three SNPs in the *IL-9 gene,* three SNPs *in the IL-4Rα gene* were obtained by using multiplex PCR Mass ARRAY matrix MALDI-TOF mass spectrometry;. For the SPT reactivity, out of the 14 allergens, seven showed a significant hypersensitivity in the majority of asthmatics, including; house dust mites (HDM), mixed moulds, D. Peterenyssinus (DP), mixed ragweed, grass pollens, and cat hair. The strongest sensitizer was HDM (P = 0.001). The analysis of SNPs for association with SPT reactivity revealed the following; the SNPs in *IL-13* in addition, *IL-4Rα* showed evidence association with SPT reactivity symptoms, but SNPs in the *IL-4 gene* associated with marginally significant (P = 0.05). An interaction of (A−646G) allele of *IL-13* with minor alleles of *IL-5, IL-9, and IL-4Rα genes*, showed a significant association with reactivity SPT. Asthmatics showed hypersensitivity to common aeroallergens, the more prevalent allergen was mixed moulds; the most significant one was HDM. More reactive alleles associated with skin reactivity were; (A-G) of the IL-13 *gene* and (G-A) of the IL-4R *gene.* An interaction of the *IL-13 gene* with *IL-5, IL-9*, and *IL-4Rα genes*, have a significant association with reactivity SPT. There is a correlation between the *IL-13 gene*, atopy, and asthma in Sudanese.

Keywords: Allergic Asthma, Allergens, Skin-Prick Hypersensitivity, Interleukin, Gene, Polymorphism, 5q31-q33, 16p11

1. Introduction

Allergic asthma is a complex disease that results from interactions between multiple genetic and environmental factors, the strongest risk factor in the etiology of asthma is atopy [1]. The knowledge of molecular genetics helps to understand the complex mechanisms of this disease [2]. It is characterized by the development of a persistent

inflammatory process of theT-Helper2 (TH2)-type triggered by exposure to certain inhaled allergens, which in susceptible individuals activates the airway epithelium and dendritic cells (DCs), leading to the synthesis of specific IgE antibodies [2]. These cells include cells of the first physical barrier; epithelial cells, mainly professional antigen-presenting cells (APC); DCs, T-helper (Th) cells, and B cells. Together, these cells form the process of allergic sensitization, which is triggered by exogenous allergenic molecules produced by human organs exposed to these molecules. In this interaction, the intrinsic properties of the exogenous proteins and environmental factors certainly play a role, but the host's immune factors play a decisive role in explaining why any individual exposed to such allergens does not develop an allergic effect [3, 4]. An allergen exposure triggers the activation of bone marrow-derived and non-bone marrow-derived cells of the innate immune system, initial exposure to allergens results in stimulated Th2 cell-dependent of the immune response that mediates the production of IgE and cytokines. Re-exposure to the allergen activates mast cells, which release mediators such as histamines and leukotriene that recruit other cells, including Th2 cells which mediate the inflammatory response in the lungs [5]. Allergic immune responses are characterized by IgE, eosinophilia, and T-cell responses producing *IL-4, IL-5,* and *Il-13*. The *IL-4* and *IL-13* are key cytokines implicated in allergic diseases, although *IL-9* is also associated with allergic inflammation [6]. The cytokines *IL-4* and *IL-13* share many structural and functional similarities, as well as the receptor *IL-4Rα gene*, located on chromosome 16p11 [7]. *IL-13* has been suggested a key cytokine responsible for peripheral inflammation, while *IL-4* can only have a central effect, one would expect that *IL-13* may be a more important mediator of Th2 responses in the skin than *IL-4* [8]. The predisposition to allergies is the result of a complex multi-factorial interaction of genes and the environment, although many individuals are known to be predisposed to develop allergic reactions to substances that would not normally trigger an immune response; these atopic individuals are thought to be genetically predisposed to develop hypersensitivity to allergens such as pollens and perfumes [9]. Genome-wide association studies (GWASs) [10], twin and family studies have investigated allergen sensitization [11] and self-reported environmental allergy [12]. Genetic variants may influence the associations between air pollution and asthma [1]. Studies have reported that, single-nucleotide polymorphisms (SNPs), in IL-4 and IL-13 genes, are associated with allergic responses [13, 14]. Studies reported, SNPs in IL-4 were associated with asthma and allergic rhinitis (AR) [15, 16]. A correlation has been found between the *IL-13* and *IL-4* genes with clinical atopy [17]. Although IL-13 is over-expressed by different cell types in the skin of atopic dermatitis (AD)[18]. Genetic studies of food allergy (FA) use a candidate gene approach to improve understanding of the genetic mechanism of FA. The *IL-13* polymorphism rs1295686 (in complete linkage disequilibrium with functional variant rs20541 is associated with FA in two independent cohorts [19]. Several candidate genes for peanut allergy (PA) have been reported [20], including mutations and genetic variants of the *filaggrin gene* (FLG). The HLA-DR and HLA-DQ regions were associated with FA in European ancestry [21]. In addition, GWASs of FA were conducted in the US to identify PA including; specific subtypes peanut, milk, and egg [21]. However, *IL-9* production and genetic susceptibility can lead to severe food allergies, and innate lymphoid cells type 2 (ILC2s). Th cells may also serve as alternative cellular sources of *IL-9*, which is a factor in the development of anaphylaxis [22]. Gene expression of *IL--9, IL13,* and *IL-31* was increased in children developing asthma and allergic sensitization to cockroach allergens [23]. Genetic factors may also play an important role in conferring the susceptibility to cockroach sensitization, an important risk factor for the development of asthma [24].

Aim and Objectives: we discuss the current understanding of how genetic and environmental factors are associated with allergic asthma. Studied the association of polymorphisms; *IL-4 rs2070874, IL-5 rs206981, [rs2069743, rs20541*] in *IL-13, [rs31563, rs2069885, rs1859430*] in *IL-9*), on chromosome 5q and, (rs2057768, rs1805010, rs1805015) in *IL-4R gene* on chromosome 16p, with, exposure to triggers (skin hypersensitivity) contributing to asthma in Sudanese families.

2. Material and Methods

2.1. Subjects

A case pseudo-control association study was conducted, in 70 Sudanese families, including 290 subjects (7 to 65 years of age), comprising of 150 asthmatics and 140 non-asthmatics. The diagnosis of asthma was based on clinical symptoms and the criteria of the Global Initiative for Asthma *http://ginasthma.org/2021-gina.* Individuals and family histories of respiratory symptoms and, exposures to different aero allergens were assessed by questionnaires. The patients with a positive family history of asthma and/or any other allergic condition like allergic rhinitis, atopic dermatitis, and eczema were selected. All patients and controls signed an informed consent showing their agreement to participate in this study. Asthmatic status was confirmed by PFTs, Blood was collected from all subjects for DNA genotyping.

2.2. Skin Prick Test (SPT)

Asthma guidelines or algorithms generally recommend specific IgE or skin prick testing for aeroallergen sensitization in asthmatic patients [3]. For confirmation of the presence of an allergy, the most common diagnostic procedures available are the IgE-specific test, a food challenge test, and SPT [25]. A skin test for responsiveness to fourteen common allergens was examined, using a standard allergen extract panel that was commonly used for asthmatic patients in Sudan (NELCO laboratories, USA). Histamine and saline were included as positive and negative

controls respectively. The recommended method of prick testing includes the appropriate use of specific allergen extracts, positive and negative controls, and interpretation of the tests after 15–20 minutes of application. Positive results were denoted by a mean wheal diameter of >3 mm above the negative control. Sensitization to at least one of the aeroallergen was recorded as a positive reaction to SPT. Atopy was defined as a positive response to 1 or more of the following; house dust mite, cat, or grass pollen [26].

2.3. Genotyping

Genomic DNA: The nucleotide positions in this study are given relative to the translation start site Table 1. The reference sequences used were retrieved from *www.ncbi.nlm.nih.gov/genbank*.

Genotyping was carried out using a multiplex PCR (SEQUENOM Mass ARRAY matrix MALDI-TOF mass spectrometry), (Sequenom Inc., San Diego, USA), Primers for PCR and single base extension were designed by using the Assay Designers soiwDre version 3.0 (Sequenom) Table 1. Genotype calling was performed in real-time with Mass ARRAY RT soiwDre version 3.0.0.4 and analyzed by using the Mass ARRAYTyper soiwDre version 3.4.

Table 1. *The gene symbol, Db SNP, alleles for each SNP and, sequences of primers.*

Gene/SNP	Ancestral Allele/ location	Position	Forward- PCR Primer	Reverse- PCR Primer	Extension primers (probes)
IL-4 rs2070874 (C-33T)	Promoter	132009710	ACGTTGGATGTGCATCG TTAGCTTCTCCTG	ACGTTGGATGGAGGTGAG ACCCATTAATAG	GCTTCTCCTGATAAA CTAATTG
IL-5 rs2069812 (C-746T)	Promoter	131879916	ACGTTGGATGCCTGCTG CTCATGAACAGAA	ACGTTGGATGTTGGGCAC CTTTCCCATTGA	ACGCTCATGAACAG AATACATA
IL-13 rs20541 (A-110 G)	Exon 4	131995964	ACGTTGGATGCCAGTTT GTAAAGGACCTGC	ACGTTGGATGTGATGCTTT CGAAGTTTCAG	CAATTTTTCGCGAGG GAC
IL-13 rs2069743 (A-646G)	Promoter	131993275	ACGTTGGATGCACTGTG AGAGGGATTGTCA	ACGTTGGATGCCCAGAAA GACCTCTGAATC	GGGATTGTCAAAGT TCAGA
IL-9 rs1859430 (C-T)	Intronic	135230513	ACGTTGGATGTCTGAGG TTGTGAGTGGAAG	ACGTTGGATGGAACTTTC CTGCCACCCATC	GGCCACTGCCCACT A
IL-9 rs2069885 (C-T)	Exon 5	135228165	ACGTTGGATGCATCCCC ACAGTATTTTTCC	ACGTTGGATGACTCTTCA GAAATGTCAGCG	CCATGCAACCAAAC CA
IL-9 rs31563 (A-4091G)	promoter	135235606	ACGTTGGATGCTTTGAT ACCCCCTCATTAC	ACGTTGGATGTTAGCAAG GGTAAAGGCCTG	CCTCATTACTACCCT CC
IL-4R 2057768 (G-A)	promoter	27322095	ACGTTGGATGTCAAGTT CCTGCCCAAGATG	ACGTTGGATGTCTTGGTGC CTTTGGACCTG	TTTATCTGTGACTGC TCC
IL-4R rs1805010 (G+4679A)	Exon 4	27356203	ACGTTGGATGACGTCAT CCATGAGCAGGTG	ACGTTGGATGTGTGTCTGC AGAGCCCAC	GCCGCCCTTGTTCTC AGGGA
IL-4R rs1805015 (T+22656C)	Exon 10	27374180	ACGTTGGATGAGAGACG CCCCTCGTCATC	ACGTTGGATGTCTCTGGG ACACGGTGACTG	AACGCAGCTTCAGC AAC

2.4. Statistical Analysis

Chi-square tests were used to test the association of SPT among cases and controls, these tests were performed using multiple logistic regression analysis to obtain the adjusted odds ratios (ORs) and, 95% confidence intervals (CIs). A significant departure from the Hardy-Weinberg equilibrium (HWE) for all SNPs was estimated using Haploview 4.2 software *(www.broadinstitute.org/haploview/haploview)*. Significant differences in genotype and allele frequency between cases and controls were assessed using χ^2 analysis. Interaction analyses were performed both between genes and between genes with SPT reactivity. This was done to investigate if the risk effect of one variable is dependent on the other. A multiple logistic regression model was used to test for interactions by adding an interaction term between genotype and phenotypes investigated. Adjustment for the covariates, all regression analyses were performed using the statistical software package *Plink version 04 http://pngu.mgh.harvard.edu/purcell/plink.* A value of $p < 0.05$ was considered significant. The study was approved by the Research Ethics Committee of The National Ribat University. Khartoum, Sudan.

All participants were informed about the objectives and the need for this study; self-confidentiality was assured and their written consent to participate was obtained before being involved in the study.

3. Results

3.1. SPT Findings

A total of 290 subjects comprised of 150 asthmatics and 140 non-asthmatics were included in this study. The duration of asthma status was 5 years in asthmatic patients. Out of the 14 allergens tested, seven of them showed significant reactivity to SPT. Table 2. Exposures to overall allergens were found to be significant among asthmatics in comparison with non-asthmatics (P = 0.001). 64.2% of subjects were sensitized to three or more allergens. The strongest allergen among asthmatics was HDM, (P = 0.001), and the highest exposure allergen among cases was found to be mixed moulds (82%) Table 2.

Table 2. *Skin reactivity allergens in asthmatics and non-asthmatics.*

Allergens	Asthmatics (n=150)	Non-asthmatics (n=140)	P –value
HDM	68.7% (103)	43.5% (61)	0.001
D P	64.6% (97)	31.4% (44)	0.001
Mixed moulds	82% (123)	53.5% (75)	0.01
Grass pollen	44% (66)	26.6% (40)	0.01
Mixed rag-weed	18% (27)	7% (10)	0.01
Cat hair	35.6% (55)	21.4% (30)	0.02
Mixed feathers	22% (33)	12% (17)	0.05
Mixed flower	44% (66)	26.5% (40)	0.06
Cockroach	30% (45)	21% (30)	0.16
Mosquito	28.6% (43)	20% (28)	0.17
Dog epithelium	23% (34)	18.5% (26)	0.51
Peanut	22% (33)	19% (27)	0.52
Goat dander	12% (18)	9.2% (13)	0.54
Mixed trees	16.6% (25)	13% (18)	0.96

3.2. Genetic Association with Hypersensitivity SPT

Among the investigated SNPs, only *IL-13 rs2069743* (P = 0.0273) and *IL-4R rs2057768* (P = 0.025), showed a deviation from Hardy-Weinberg equilibrium. There are no significant differences in genotype distributions and allele frequencies between cases and controls for other SNPs. Genetic association of SNPs with reactivity SPT showed; evidence associating for *IL-13 rs2069743* (P = 0.003) and *IL-4 rs2070874* showed a marginal association among asthmatics (P = 0.05), Significant gene-gene interaction was found between the (A−646G) allele of *IL-13 rs2069743* and minor alleles of *IL-5, IL-9,* and *IL-4Rα genes* with SPT reactivity Table 3. The *IL-4Rα rs2057768* showed evidence associated with SPT reactivity symptoms when interacted with minor alleles of *IL-4Rα rs1805015, IL-5 rs2069812* and *IL-9 rs2069885* Table 4.

Table 3. *Gene-gene interaction between the G minor allele of IL-13 rs2069743 and minor alleles of IL-5, IL-9 and, IL-4Rα genes with SPT reactivity.*

Associated allele-(A−646G)	OR	P-value	Condition
G	2.3	0.003	-
G	2.03	0.03	when interacted with T allele of *IL-5 rs2069812*
G	2.07	0.02	when interacted with Tallele of *IL-9 rs1859430*
G	2.09	0.02	when interacted with A allele of *IL-9 rs31563*
G	2.04	0.03	when interacted with T allele of *IL-9 rs2069885*
G	2.08	0.02	when interacted with A allele of *IL-4Rα rs2057768*
G	1.97	0.04	when interacted with A allele of *IL-4Rα rs1805010*
G	2.04	0.03	when interacted with C allele of *IL-4Rα rs1805015*

Table 4. *Gene -gene interaction between the A allele of IL-4Rα rs2057768 and minor alleles of IL-5, IL-9 and, IL-4Rα genes with SPT reactivity.*

IL-4Rα rs2057768 associated allele	OR	P-Value	Condition
A	0.38	0.05	when interacted with C allele of *IL-4Rα rs1805015*
A	0.41	0.04	when interacted with T allele of *IL-5 rs2069812*
A	0.14	0.05	when interacted with T allele of *IL-9 rs2069885*

4. Discussions

Given our understanding of the complex interplay between various genes and environmental factors and their potential to modify asthma outcomes, the study of gene-gene interactions seems essential. Although numerous studies of specific gene-environment interactions have defined the pathophysiological phenotype more closely and have begun to identify a series of key genes that may be involved in the development of allergic diseases [13, 14]. The present study demonstrates gene-environment interactions, as well as gene-gene interactions effects between genetic variants of the *IL-4, IL-5, IL-13, IL-9,* and *IL-4R genes,* which are associated with SPT responsiveness and influence atopy in Sudanese families. The role of these genes in allergic disorders and skin sensitization has been well documented in several studies [1, 23]. Numerous studies have confirmed that individual polymorphisms do not always affect disease susceptibility; however, the combined effect of many SNPs with mutant alleles in different genes can greatly increase disease susceptibility. Our study aimed to determine if there is a gene–gene interactions (epistasis) between genetic variants in 5q31-q33 and 16p associated with allergic sensitization. In this study, when individual SNPs were analysed, none of the SNPs except *IL-13 rs2069743* were individually associated with SPT reactivity. This study demonstrates that the IL-13 SNP confers a significant genetic risk in asthma patients. the G allele of IL-13 rs2069743 has been shown to be associated with skin hypersensitivity among the highly "exposed" subjects; interestingly, an association of genetic variants and environmental components was observed with combined

SNPs in *IL-13, IL-5, IL-9,* and *IL-4R genes* IL-13, although there was a significant interaction between SNPs of these genes and the G allele of the IL-13 *gene,* which is associated with skin reactivity and asthma development, as previously described in numerous studies. In the British National Survey of Health and Development, a longitudinal birth cohort found that *IL-13 rs20541* and rs1800925 were each significantly associated with self-reported asthma and allergy [27]. However, gene-gene interaction was reported in the Dutch population [28], whites [29], African Americans [30], and Sudanese [31]. The minor alleles G, for both rs1805010 and rs1801275 SNPs in *IL-4Rα gene*, were associated with asthma predisposition and allergic disorders in Saudi asthmatic patients [32]. However, the present study detected gene-gene interactions related to asthma and SPT reactivity in Sudanese families, which was confirmed in several studies; a study in Polish children found that, *IL-13 rs20541* and *IL-4 rs2243250* were associated with SPT reactivity [17]. The combination of *IL-13 rs2054*1with *IL-4R rs1805011*, and *IL-13* C-1111T with *IL--4R rs1805013* produced a higher risk for asthma and skin test responses than individual effects [33]. On the other hand, the importance of *IL-4, IL-13*, and their associated receptors has been reported in AD [34, 35]. Another study described an association between *IL-13* (C−1112T) and *IL-4* (G+3017T) SNPs, and mould allergy susceptibility in African Americans [36]. Genetic polymorphisms of *IL-13* (C–1112T, *IL-4RA* (I50V, *IL-5* (C–746T), and Adrenergic Receptor beta-2 (ADRB2) (Q27E, R16G) was strongly associated with olive pollen allergen and asthma development [37]. In addition, *IL-9* has been linked to atopy [38], although, the *IL-9* gene is associated with specific IgE against HDM in asthmatic patients [39]. However, increased expression of IL-9, IL-13, and IL-31 was associated with the development of asthma in African American children sensitized to cockroaches [23]. In a study conducted in children, *IL-9* was expressed as PA, followed by *IL-5,* and then *IL-13* [40]. A significant additive interaction between mold exposure and the rs7216389 SNP in childhood asthma was observed [41]. Epistasis between immune *IL-4R* and skin FLG regulatory genes synergizes in the development of allergic sensitization [42]. In this study, asthmatic patients with a positive family history were more sensitive to allergens (64.2%) than those without a family history. Important allergen contributors causing significant reactivity in asthmatic patients were HDM, DP, mixed mold, pollen, mixed weeds, and cat hair. The most common allergens were mixed mould, with 82% having overt reactivity. Our findings are consistent with two previous studies indicating the most prevalent allergen was mould [43]. The allergens most associated with allergic asthma are; house dust mites [44], cockroaches, and Cladosporium species [45]. In South Jordan, children sensitized to the most common allergens that trigger asthma were DP, pollens, and cat fur [46]. But unlike a study in Angola, children were less sensitive to airborne allergens, the most common being dust mites, cockroaches, and fungi. [47]. This study showed a prevalence rate of sensitivity to HDM 68.7%, DP 65%, grass pollen 44%, cat hair 35%, cockroach 30%, dog epithelium 23%, peanut 22%, and ragweed 18%, which are similar to studies in some allergens but differ in others; a prevalence rate of sensitivity to HDM 72.2%, cockroach 54.8%, mixed grasses 69%, and peanut 29.4% [48]. The most common sensitizing allergens identified by Korean SPTs; were house dust mites (40.9%), followed by the cockroach (23.6%%), and dog dander (8.2%) [49]. Studies reported that the most sensitive aeroallergens are DP (22.5%), D farinae (21.3%), Timothy grass, and Alternaria alternans (20%) [50]. HDM >50%, (cockroach, cat, and dog) 20%, ragweed, mixed grass <10% [51]. HDM 65.3%, cockroach 19.2% [45]. However, our results differ from the rate reported in these studies, probably due to different environments, genetic factors, and varying degrees of exposure to these allergens. However, sensitization to allergens can be a real risk factor for the development and severity of allergic diseases in the USA and Europe [49]. Zimbabwe [52]. Studies have reported that sensitization to common allergens such as HDM and cockroach antigens may further increase the risk of asthma [45]. Another study conducted by a cohort in the USA shows that sensitivity to cockroaches is associated with the development of allergies [53]. This is the first study to evaluate aeroallergen sensitization on asthma patterns and highlight the importance of gene-gene interaction in genetic association studies among Sudanese. Although considering the gene-environment interaction is important and, should be incorporated in future studies to determine whether the risks associated with environmental exposures are the same for people with different genetic susceptibilities in Sudan.

5. Conclusion

This is the first study to assess the impact of aeroallergen sensitization on asthma patterns and highlights the importance of intragenic interactions in genetic association studies in Sudanese., Although consideration of gene-environment interactions is important and should be included in future studies to determine if the risks associated with environmental exposures are the same for people with different genetic susceptibilities in Sudan. Epistasis between SNPs in *IL-13, IL-9, IL-5, IL-4R,* and SPT reactivity was observed, and might confer a higher risk for asthma-related traits than single risk alleles. Therefore, our findings suggest these genes on 5q31–33 and 16p12 could be susceptible genes to atopy in Sudanese. The most important polymorphisms associated with skin reactivity were; rs2069743 in the IL-13 gene on 5q31–33 and rs2057768 in the IL-4R gene on 16p12. There is strong evidence that; an *IL-13* variant is more significant than the other variants for SPT reactivity. Asthma patients in Sudan showed hypersensitivity to common aeroallergens, including; mixed molds, HDM, DP, and grass pollen, which are the most common aeroallergens. Finally, it seems there is a link between environmental exposure and epigenetics, which warrants further investigation.

Data Availability

The data used to support the findings of this study are included.

Conflict of Interest Statement

All the authors do not have any possible conflicts of interest.

Abbreviations

IgE	Immunoglobulin E
PFT	Pulmonary-FunctionTest
SPT	Skin prick test
SNP:	Single nucleotide polymorphism
HDM	House Dust Mites
DP	D. Peterenyssinus
Th2	T-helper 2
DCs	Dendritic Cells,
APC	Antigen -Presenting Cells
GWASs	Genome-wide association studies
AR	Allergic Rinities
AD	Atopic Dermatitis
FA	Food Allergy
PA	Peanut Allergy
FLG	Filaggrin gene
ILC2	Type 2 Innate Lymphoid Cell
HWE	Hardy-Weinberg equilibrium
ADRB2	Adrenergic Receptor beta-2

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