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Role of interleukins 12B and 17A genetic variation in house dust mites allergy

Asmaa Mostafa Ammar[1*], Elham Abbas EL. Zayyat[1], Amira EL. Saady Khayyal[1], Dina Mamdouh Hamdy[1], Aya EL-Gendy[2] and Noha Abdel Fattah Elleboudy[1]

Abstract

Background: The house dust mites (HDM) constitute a major cause of allergic diseases all over the world. Genes encoding interleukins 12B and 17A which determine the course of T cell-mediated immune response are prime candidates as allergic disease susceptibility. The purpose of this study was to evaluate whether a single-nucleotide polymorphisms (SNP) of interleukins 12B + 1188A/C (rs3212227) and 17A −197G/A (rs2275913) confers susceptibility to HDM allergic diseases. Through a case-control study, 120 subjects served as 60 dust mites' allergic patients and 60 healthy non-allergic controls. Total immunoglobulin (Ig) E level, eosinophilic count, serum interleukins 4, 10, 12B, and 17A levels for the studied subjects were measured. Then, genotyping of single-nucleotide polymorphisms (SNPs) at +1188A/C for IL12B and −197G/A for IL17A gene were conducted using restriction fragment length polymorphisms (RFLP-PCR).

Results: The present study showed that in HDMs' allergic subjects there was a significant increase in IL12B (+1188 A/C) and IL17A (−197 G/A) genotype variants compared to that of the controls. There was a significant increase in total IgE levels, eosinophil counts, and the levels of both IL-4 and IL-17A, while IL12B was significantly lower in patients compared to that of the controls. There was no significant difference in IL-10 levels between patients and controls.

Conclusion: Our findings indicate that IL12B (+1188 A/C) and IL17A (−197G/A) might be associated with an increase in the susceptibility to dust mites' allergic patients.

Background

Allergic diseases are now one of the top conditions in the twenty-first century requiring a lot of effort for prevention and control. Allergic diseases are still largely unclear despite abundant research. Up to 25% of the world's population is suffering from allergic conditions such as asthma, allergic rhinitis, and allergic dermatitis [1]. Allergy is an IgE-mediated disease caused by allergens sensitization. These previously mentioned allergic diseases are the most common familial IgE-related diseases [2]. The house dust mites (HDM) are the main cause of allergic stimulation in patients with atopic conditions [3, 4].

Allergy is caused by an interaction between environmental and genetic factors [5]. Atopy development depends on multiple genes that affect the expression of disease. Various genome-wide searches have related atopy development to specific autosomal chromosomal regions [6]. Among populations, genes that predispose individuals to allergy are not consistent. In this regard, Th1 and Th2 cytokines and their gene polymorphism seem significant. These cytokines genes polymorphisms could affect the transcription of the genes, causing inter-individual variations in the production of cytokines [7].

The existence of a single-nucleotide polymorphism (SNP) at 3′UTR +1188 A/C is linked to the production of IL-12B in various pathologies affecting the Th1/Th2 balance [8], susceptibility and severity of different types diseases [9, 10]. Polymorphism of the gene IL-17A

* Correspondence: Asmaa.mostafa273@gmail.com
[1]Medical Parasitology Department, Ain-Shams University, Ramsis Street, Abbassia Square, Cairo, Egypt
Full list of author information is available at the end of the article

(rs2275913 G/A) has a significant role in the activity of interleukin by modifying the role of cytokine and regulation of its expression [11]. However, the association between such polymorphisms and the immune response in an allergy to dust mites was not explored. We studied the polymorphic region in IL-12 p40 (3′UTR +1188 A/C) and IL17A (−197G/A) and the potential correlation of dust mites' allergy susceptibility in Egyptian patients.

The initial exposure to HDM allergens in predisposed individuals contributes to the activation of the allergen-specific T-helper 2 (Th2) cells and the synthesis of IgE. Allergy has been associated with several cytokines involved in the inflammation and immunoregulation pathways [12].

Interleukin (IL)-4 is a pro-inflammatory interleukin that has long been associated with allergic disorder pathogenesis. This cytokine plays a role in the onset and propagation of allergic inflammation [13]. IL-10 is a pleiotropic anti-inflammatory interleukin that functions mainly by suppressing the pro-inflammatory cytokines [14].

IL-12 is a heterodimer cytokine formed by a 35 kDa chain (p35 or IL-12A) and a 40 kDa chain (p40 or IL-12B). IL-12B is encoded by the IL12B gene located on 5q31–33 and because of its pro-inflammatory and immunoregulatory activities it might be an important functional candidate gene for inflammatory diseases. IL12B induces the production of interferon-γ (IFN-γ), favors the differentiation of T helper 1 (Th1) cells, and it is a connecting point for innate and adaptive immunity [15].

The IL-17 cytokine family includes six members (A–F). IL-17A is a proinflammatory cytokine that upregulates several cytokines and chemokines, leading to the recruitment of neutrophils to sites of inflammation [16]. Several studies have shown a strong association between IL-17A and neutrophil inflammation, involving IL-17A as a possible candidate gene to predict allergy susceptibility [17, 18].

Methods

Ethics approval and consent to participate

The study had the required approval from the ethical committee of the Faculty of Medicine Ain Shams University. Written informed consent has been obtained from all participants. Patients found allergic to dust mites have been referred for immunotherapy whenever accepted by them.

Study population

The population of this study included a total of 120 subjects; dust mites' allergic patients (n = 60) and controls (n = 60). We enrolled dust mites' allergic patients, from the Allergy and Clinical Immunology outpatient clinic in the tertiary teaching hospital (Ain Shams University Hospitals, Egypt). The criteria for study inclusion were adults patients over 18 years of age, those who were diagnosed with dust mites allergic diseases (asthma, allergic rhinitis, and allergic dermatitis) by an allergy specialist by history, physical examination, and positive skin prick test results using prepared mixed dust mites crude extracts. Asthma was diagnosed based on GINA guidelines, 2018 [19] with typical symptoms and signs, including wheezing, shortness of breath, chest tightness, or cough. Allergic rhinitis was based on ARIA guidelines, 2010 [20] with symptoms and signs of rhinorrhea, nasal obstruction, and itching sensation or sneezing. We assessed if the symptoms of allergic diseases were aggravated after exposure to mites (e.g., after bedding, cleaning a closet, or sleeping on an old bed). Atopic dermatitis was diagnosed when atopy was associated with chronic relapsing eczematous dermatitis with pruritis [21].

Controls criteria included: adults over 18 years of age with no symptoms or history of allergic diseases, negative skin prick test results for prepared mixed dust mites crude extract. Patients on specific allergen immunotherapy, pregnant women, patients on corticosteroids, oral antihistamines, or any other drugs that inhibit skin prick test reactions, and patients with positive dermographism were excluded from the study.

Preparation of dust mites crude extract

Mixed crude mite extraction was carried out as follows: the mite extract was prepared from mixed species of dust mites by extracting pure mites at a final concentration of 1: 10 w/v in Coca solution (2.5 gm sodium bicarbonate (NaHCO3), 5 gm sodium chloride (NaCl), 5 gm phenol crystals and 1000 ml sterile water for injection) under a laminar flow cabinet with sterile technique. The mixture of mites and Coca solution was magnetically stirred at room temperature for 1 h. The solution was filtrated twice, first through Whatman No. 1 filter paper (11μm), and then pass through a 0.2-micron filter using the Vacuum filtering apparatus. The mite extract was stored at 4 °C and was used within 3 months. Mixed crude mites extraction was carried out at the Allergy laboratory at the Allergy Medicine Unit, Internal Medicine Department, Ain-Shams University hospital, Egypt [22].

Skin prick test

Skin prick tests were carried out using prepared mixed dust mites crude extracts, as recommended by the European Academy of Allergy and Clinical Immunology. Saline was used as control negative and histamine as control positive. Skin testing was considered positive if a mixed crude extract of prepared dust mites elicits a wheal reaction of > 3 mm in diameter after 15 min. Before clinical evaluation, none of the participants received antihistamines, systemic, or topical corticosteroids 3 weeks before [23].

Blood samples collection

A 6-ml blood sample was taken and divided into 2 portions: whole blood for DNA extraction and eosinophil Counts. Serum samples for total levels of IgE and interleukins measures [24].

Eosinophil count and total serum immunoglobulin E determinations

A complete blood picture was performed to determine the eosinophilic count in the peripheral blood. Total IgE level was measured by ELISA using the Manufacturer's protocol based on the BIOS IgE Quantitative Kits (South San Francisco, USA). The Assay sensitivity was 5.0 IU/ml for total IgE.

Detection of serum interleukins

The serum levels of IL-4, IL-10, IL12B, and IL-17A were measured simultaneously with the Magnetic Luminex Multianalyte technology. The test was conducted as instructed by the manufacturer (R&D Systems, Bio-Techn Assay Kit, USA). Data were analyzed using the Luminex Labscan 100 Multiplexing System (USA, Model No: LX10007073403). The reading for the concentration of each cytokine was detected by the instrument as the mean fluorescence intensity (MFI). These results were subsequently converted from a set of standards that were running concurrently in the assay to pg/ml of cytokine-dependent on the MFI values.

DNA extraction

Genomic DNA was extracted from 200 μl of whole blood using the QIAamp ® DNA Blood Mini Kit (Qiagen, Germany) according to the Blood and Body Fluid Spin Protocol in the accompanying manual. After the DNA was extracted, the samples were labeled and stored at − 20 °C.

IL12B and IL17A polymorphisms

The genotypes of IL-12B rs3212227 and IL-17A rs2275913 were analyzed using the polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method according to Youssef [25] for IL12B and Hammad [26] for IL17A. Genomic DNA from the cases and controls were subjected to PCR analysis of the IL-12B and IL17A genes. The sequence of primers (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) used are presented in Table 1

Table 1 IL-12B and IL-17A primers sequence

IL SNP	Primer sequence
IL-12B rs3212227	forward: 5′-TTCTATCTGATTTGCTTTA-3′ reverse: 5′-TGAAACATTCCATACATCC-3′
IL-17A rs2275913	Forward 5′-CAG AAG ACC TAC ATG TTA CT-3′ Reverse 5′-GTA GCG CTA TCG TCT CTC T-3′

The reaction volume was 25 ul: 5 ul extracted DNA, 12.5 ul Hot Star-Taq® PCR master mix (Qiagen, Germany), 0.5 ul of each primer, and 6.5 ul RNase-free water.

Reaction conditions were carried out in Thermo Scientific HBPXE02110 PXE 0.2 Thermal Cycler (USA) with the following cycling parameters for IL-12B, an initial 95 °C for 15 min followed by 40 cycles of 94 °C for 45 s, 43 °C for 1 min, and 72 °C for 45 s and a final extension at 72 °C for 5 min. For IL-17A, the PCR conditions included an initial 95 °C for 15 min followed by 40 cycles of 94 °C for 45 s, 58.5 °C for 1 min, and 72 °C for 45 s and a final extension at 72 °C for 10 min. Ten microliters of PCR products were evaluated in 2% agarose gel to check the PCR products at 233 base pairs (bp) for IL12B and 344 bp for IL17A Figs. 1 and 2.

RFLP analysis for IL12B was done using Taq-1 restriction enzyme (Promega, Southampton, UK). For IL17A, RFLP analysis was done using XmnI restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA). DNA fragments were resolved in 2.5% agarose gels. The results of genotyping were confirmed by randomly assaying 10% of the original specimens for replicate to exclude genotyping errors. There were no differences between genotypes identified in duplicate.

Statistical analysis

Analysis of the data was done using SPSS software (Statistical Package for the Social Sciences) version 20. Statistical presentation and analysis of the present study were conducted, using the mean, standard deviation, Student's *t* test, Fisher exact test, and Analysis of variance [ANOVA] tests.

Results

This is a case-control study that was conducted on patients selected from the outpatient clinic of Allergy and Clinical Immunology at the tertiary hospital. It included 60 patients with dust mites' allergy and 60 non-allergic persons as control. The range of age was between 19 and 50 years old. They were 72 (60%) females and 48 (40%) males. The enrolled studied groups were divided into 2 groups according to the results of skin prick tests which was considered positive if prepared dust mites crude extract elicit a wheal reaction > 3 mm in diameter after 15 min. into cases (dust mites allergic patients with positive skin prick tests) and controls (non-allergic persons with negative skin prick tests). The enrolled patients were divided into three groups according to clinical presentation into asthmatic, Allergic Rhinitis (AR), and dermatitis patients.

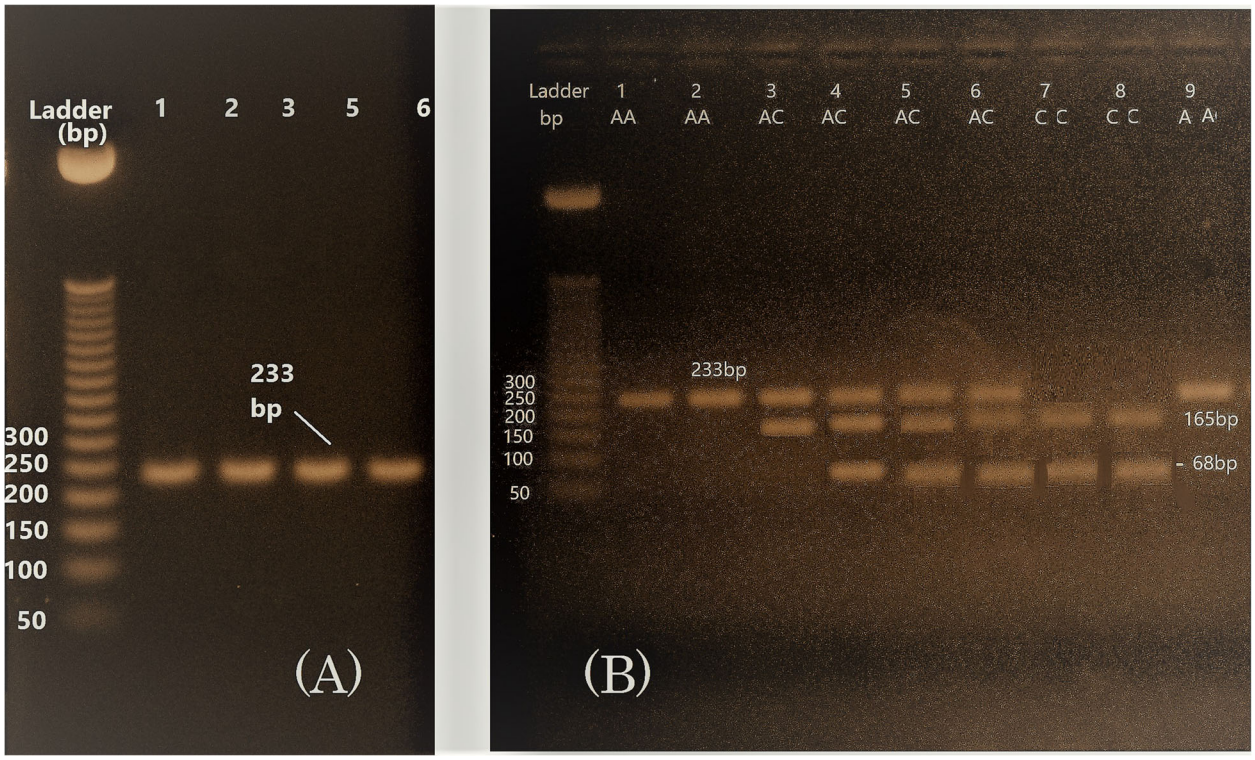

Fig. 1 Results of agarose electrophoresis of the interleukin (IL)-12B rs3212227 polymorphism. **a** Represent the PCR amplified product at 233 bp. **b** Represent the restriction fragment length polymorphism analysis reaction (RFLP). Lanes 3, 4, 5, and 6 represent the interleukin (IL)-12 AC genotype (three bands at 233, 165, and 68 bp). Lanes 7 and 8 represent the IL-12 CC genotype (two bands at 165 and 68 bp). Lanes 1 and 2 represent the IL-12 AA genotype (one band at 233 bp). The ladder is a 50-bp molecular-weight marker

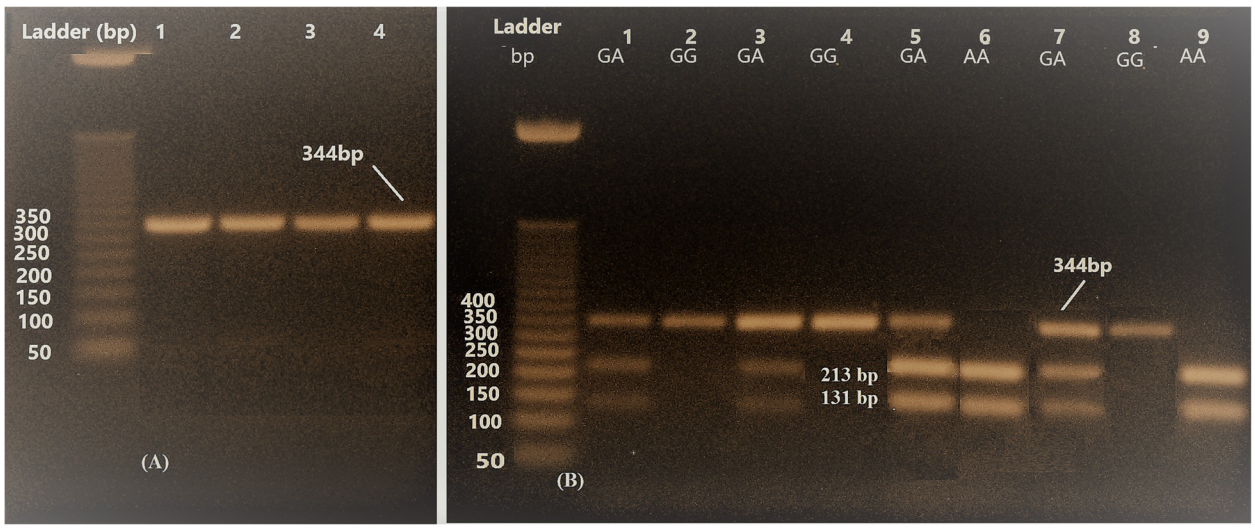

Fig. 2 Results of agarose electrophoresis of the interleukin IL-17 A rs2275913. **a** Represent the PCR amplified product at 344bp. **b** Represent the restriction fragment length polymorphism analysis reaction (RFLP). Lanes 1, 3, 5, and 7 represent the GA genotype (three bands at 344, 213, and 131 bp). Lanes 6 and 9 represents the AA genotype (two bands at 213 and 131 bp). Lanes 2, 4, and 8 represent the GG genotype (single band at 344 bp). The ladder is a 50-bp molecular-weight marker

Comparison of demographic features between the studied groups

Comparison of demographic features between the studied groups revealed that there was a statistically non-significant difference between the studied groups regarding age, gender, residence, occupation, smoking, and deal with animals (Tables 2 and 3).

Student's *t* test was used. Age is represented as mean and SD, while gender, residence, occupation, smoking, and deal with animals are represented as number and percent

Eosinophil count and total IgE levels

Peripheral blood eosinophil cell counts (PBEC) were found to be significantly higher at $p < 0.001$ in mites' allergic patients (mean value was 576.9 ± 60.03 cells/mm^3) compared to the controls group (mean value was 142.9 ± 31.0 cells/mm^3). Our results revealed a significant elevation in PBEC in asthma, allergic rhinitis, and dermatitis compared with controls (mean values for PBEC were 636.3 cells/mm^3, 576.9 cells/mm^3, and 517.7 cells/mm^3, respectively; $p < .001$ for each group)

Total serum IgE levels were found to be significantly higher at $p < 0.001$ in mites allergic patients (mean values was 276.9 ± 141.2 IU/ml) compared to the controls group (mean values was 15.5 ± 15.9 IU/ml).

Our results showed a significant elevation in total serum IgE levels in asthma, allergic rhinitis, and dermatitis compared with controls (mean values for total serum IgE levels were 342.0 IU/ml, 226.9 IU/ml, and 262.0 IU/ml respectively; $p < .001$ for each group) as shown in Figs. 3 and 4, Tables 4 and 5.

Serum levels of the studied cytokines

IL-4, 10, 12B, and 17A serum cytokines levels were assessed in dust mites' allergic patients and controls group. The level of IL-4 had a significant difference between dust mites' allergic patients compared with the controls group ($p < 0.001$). The mean value of allergic patients was (44.76 ± 6.3 pg/ml) while in the controls group was (6.72 ± 0.47 pg/ml). Our results showed a significant elevation in IL 4 levels in asthma, allergic rhinitis, and dermatitis compared with controls (mean values were 51.3 pg/ml, 41.5 pg/ml, and 41.5 pg/ml respectively; $p < .001$ for each group).

The level of IL-17A had a significant difference between dust mites' allergic patients compared with the controls group ($p < 0.001$). The mean value of allergic patients was 32.2 ± 9.2 pg/ml while in the control group was 3.31 ± 0.85 pg/ml. The results revealed a significant elevation in IL 17A levels in asthma, allergic rhinitis, and dermatitis compared with controls (mean values were 42.2 pg/ml,

Table 2 Comparison between dust mites' allergic patients and controls regarding demographic characteristics

	Control *N* = 60 (%)	allergic patients *N* = 60 (%)	*p* value
Gender			
Male	24 (40.0)	24 (40.0)	1.000
Female	36 (60.0)	36 (60.0)	
Residence			
Rural	18 (30.0)	18 (30.0)	1.000
Urban	42 (70.0)	42 (70.0)	
Occupation			
Not working	18 (30.0)	24 (40.0)	1.000
Farmer	24 (40.0)	12 (20.0)	
Worker	6 (10.0)	12 (20.0)	
Employee	12 (20.0)	12 (20.0)	
Smoking			
No	42 (70.0)	44 (73.3)	0.688
Yes	18 (30.0)	16 (26.7)	
Deal with animals			
No	18 (30.0)	24 (40.0)	0.254
Yes	42 (70.0)	36 (60.0)	
Age			
Mean ± SD	29.9 ± 8.6	31.1 ± 8.2	0.115
Range	19 ± 43	19 ± 50	

Table 3 Comparison of the studied groups regarding demographic characteristics

	Controls *N* = 60 (%)	Asthma *N* = 20 (%)	AR *N* = 20 (%)	Dermatitis *N* = 20 (%)	Fisher exact test	*p* value
Gender						
Male	24 (40)	12 (60)	10 (50)	2(10)	5.922	0.127
Female	36 (60)	8 (40)	10 (50)	18 (90)		
Residence						
Rural	18 (30)	6 (30)	6 (30)	6 (30)	.234	1.000
Urban	42 (70)	14 (70)	14 (70)	14 (70)		
Occupation						
Not working	18 (30)	6 (30.0)	8 (40.0)	10 (50.0)	4.531	0.929
Farmer	24 (40.0)	6 (30.0)	2 (10.0)	4 (20.0)		
Worker	6 (10.0)	4 (20.0)	6 (30.0)	2 (10.0)		
Employee	12(20.0)	4(20.0)	4 (20.0)	4 (20.0)		
Smoking						
No	42 (70.0)	16 (80.0)	10 (50.0)	18 (90.0)	4.073	0.301
Yes	18 (30.0)	4 (20.0)	10 (50.0)	2 (10.0)		
Deal with animals						
No	18 (30.0)	10 (50.0)	6 (30.0)	8 (40.0)	1.250	0 .889
Yes	42 (70.0)	10 (50.0)	14(70.0)	12 (60.0)		
Age					ANOVA	
Mean ± SD	29.9 ± 8.6	34.6 ± 10.3	27.4 ± 7.2	31.3 ± 7.5	> 0.05	
Range	19–43	19–50	19–43	19–40		

Age is represented as Mean and SD, while gender, residence, occupation, smoking, and deal with animals are represented as number and percent

32.2 pg/ml, and 22.2 pg/ml, respectively; $p < .001$ for each group) (Figs. 3 and 4).

On the other hand, the IL 12B level was significantly ($p < 0.001$) lower in patients than controls. The mean value of patients was 170.49 ± 50.72 pg/ml while in the control group was 264.46 ± 31.35 pg/ml. The mean values of IL12B levels were significantly lower in asthma, allergic rhinitis, and dermatitis compared with controls (144.1 pg/ml, 144.1 pg/ml, and 223.3 pg/ml respectively).

Regarding IL 10 level, there was a statistically non-significant difference ($p > 0.05$) between the studied groups. The mean value of patients was 2.8 ± 1.2 pg/ml while in the control group was 3.5 ± 0.45 pg/ml. Level of IL-10 had no significant difference in asthma, allergic rhinitis, and dermatitis compared with controls (mean values were 2.9 pg/ml, 2.9 pg/ml, and 2.6 pg/ml, respectively; $p < .001$ for each group) as demonstrated in Tables 4 and 5.

Genotype and allele frequencies of IL12B (1188 A/C)

As regards the results of PCR-RFLP for IL12B (+1188 A/C) gene polymorphism in the studied groups (whole 120 persons), the PCR product was at 344 bp. Genotyping results by the PCR–RFLP showed the existence of the 3 genotypes of the IL-12B rs3212227 polymorphism (AA, AC, and CC), and examples of these results are shown in Fig. 1. Genotypes were determined as follows, AA (233 bp), AC (233, 165, 68 bp), and CC (165, 68 bp). The homozygous genotype (C/C) was detected in 2/120 persons (1.66%) (2/60 patients (3.3%) and 0/60 persons (0%) in the control group). The heterozygous genotype (A\C) was detected in 46/120 persons (38.3%) (40/60 patients (66.7%) and 6/60 persons (10 %) in the control group). The homozygous genotype (A\A) was detected in 72/120 patients (60%) (18/60 patients (30%) and 54/60 persons (90%) in the control group).

There was a statistically significant difference between the studied groups regarding IL 12B polymorphism and alleles as demonstrated in Table 6, Figs 5 and 6. Table 7 Genotype and allele frequencies of IL12B (+1188 A/C) and IL17A (−197G/A) polymorphisms among studied groupsIL 12B AA genotype was highly significantly lower (p value ≤ 0.01) in patients than controls (Table 7, Figs. 5 and 6). On the other hand, AC and CC genotypes showed a highly significant difference in patients compared with the controls group (p value ≤ 0.01). Distribution of alleles frequency of IL12B (+1188 A/C) in all studied groups (120 persons) was as the following: the frequency of A allele was 79.2%, while the frequency of C allele was 20.8%. An allele was significantly higher in controls compared with

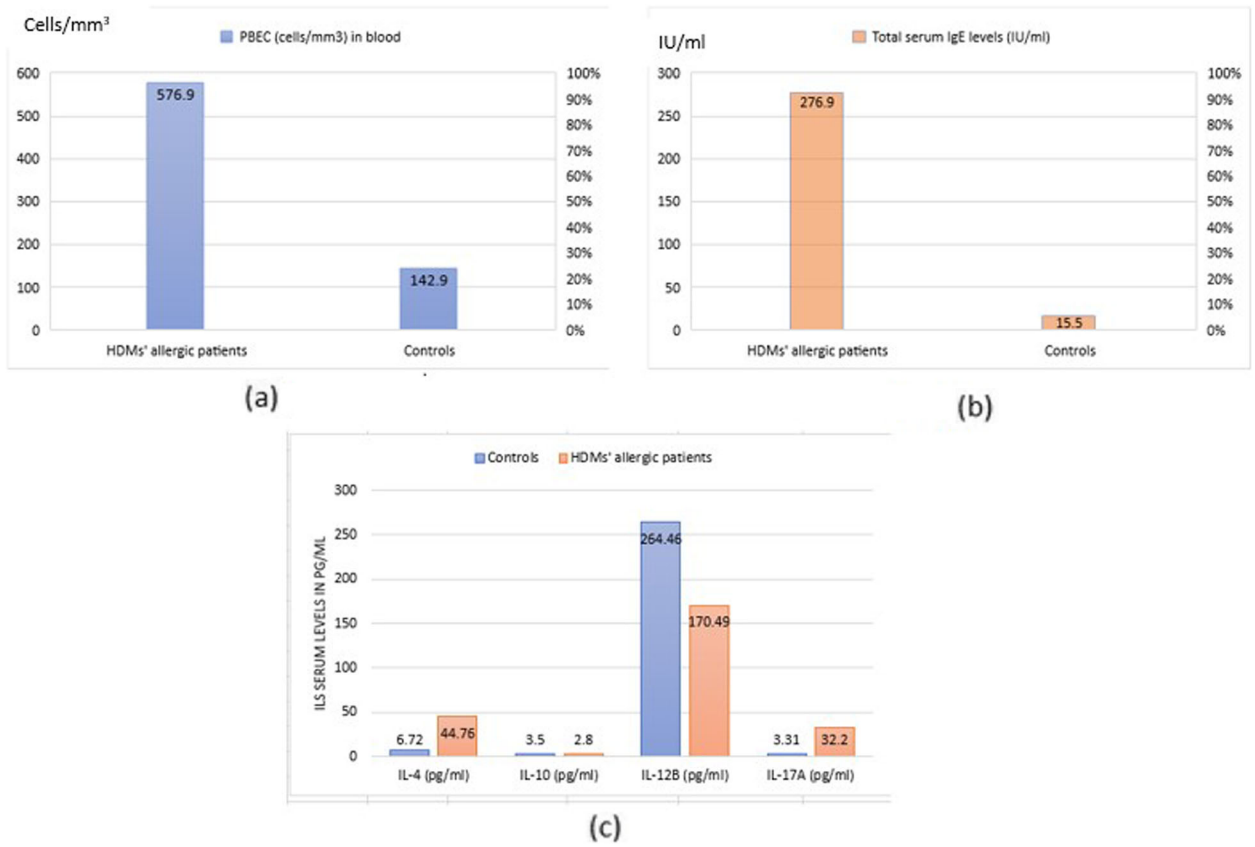

Fig. 3 **a** A graph comparing the total PBEC in patients and controls. **b** A graph comparing the total IgE levels in patients and controls. **c** A graph comparing IL4, 10, 12B, and 17A levels in patients and controls. All parameters represented as mean.

patients. On the other hand, the C allele was significantly higher in patients compared with the control group (p value ≤ 0.01).

Genotype and allele frequencies of IL17A (−197G/A) polymorphism

As regards the results of PCR-RFLP for IL17A (−197G/A) gene polymorphism in the studied groups (whole 120 persons), the PCR product was at 344 bp. Genotyping results by the PCR–RFLP showed the existence of the 3 genotypes of the IL-17A rs2275913 polymorphism (GG, GA, and AA), and examples of these results are shown in Fig. 2. Genotypes were determined as follows: GG (344 bp), GA (344, 213, 131 bp), and AA (213, 131 bp).

The homozygous genotype (A/A) was detected in 12/120 persons (10%) (6/60 patients (10%) and 6/60 persons (10%) in the control group). The heterozygous genotype (G\A) was detected in 44/120 persons (36.6%) (38/60 patients (63.3%) and 6/60 persons (10 %) in the control group). The homozygous genotype (G\G) was detected in 64/120 persons (53.3%) (16/60 patients (26.7%) and 48/60 persons (80%) in the control group).

There was a statistically significant difference between AR and dermatitis allergic patients compared with the control group ($p < 0.05$) as showed in Tables 6 and 7.

IL 17A GG genotype was significantly fewer in patients than controls. On the other hand, GA genotypes were significantly higher in patients compared with the control group ($p < 0.05$). There was a statistically non-significant difference in genotyping ($p > 0.05$) between the asthmatic group and controls. Distribution of alleles frequency of IL17A (−197G/A) in all studied population (120 persons): the frequency of the G allele was 71.7%, while the frequency of A alleles was 28.3%. G allele was significantly higher in controls compared with patients. On the other hand, A allele was significantly higher in patients compared with the control group (p value ≤ 0.01).

Genotypes of IL12B (+1188 A/C) polymorphism and its effect on IgE and IL-12B serum levels

There was a statistically non-significant difference between gene polymorphism and IgE and IL-12B serum levels as shown in Table 8.

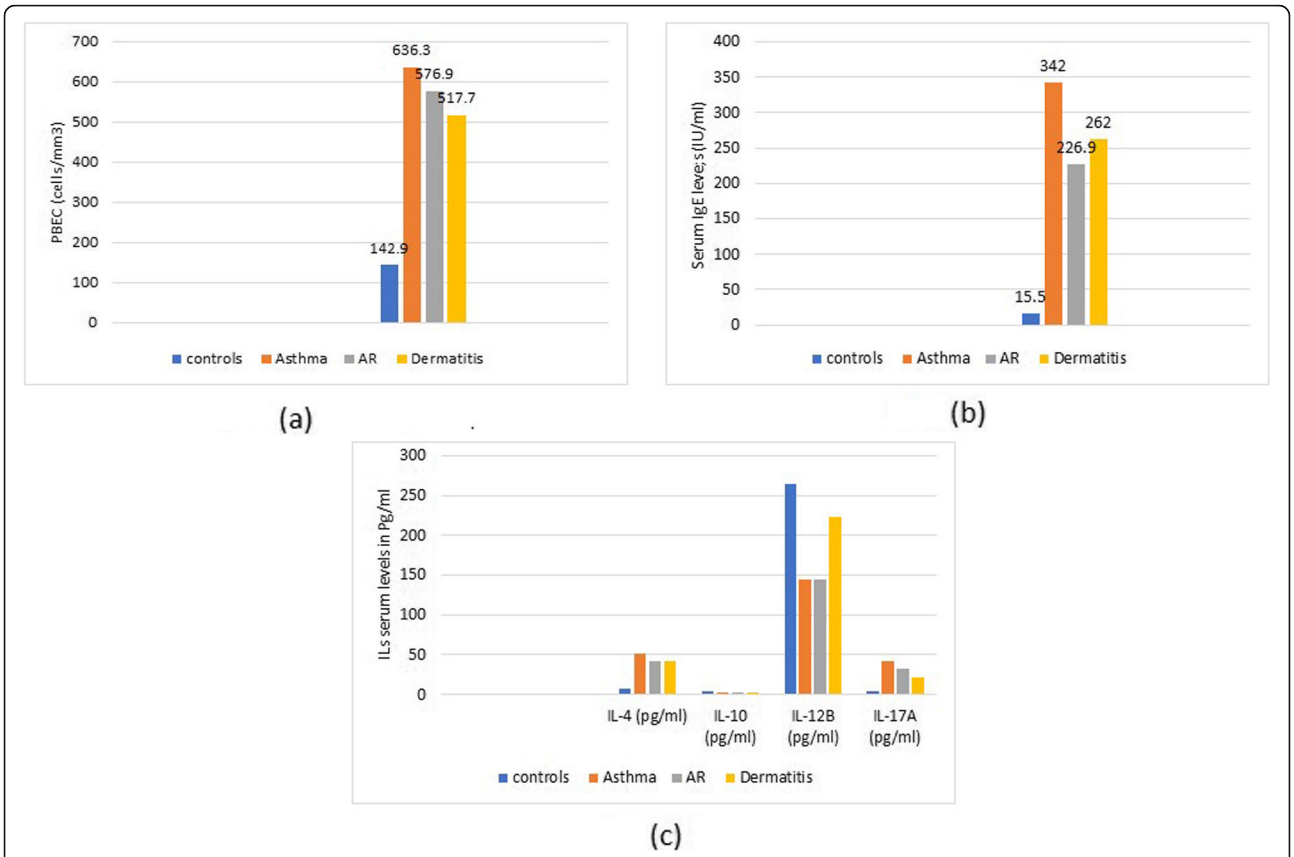

Fig. 4 **a** A graph comparing the total PBEC in patients' subgroups and controls. **b** A graph comparing the total IgE levels in patients' subgroups and controls. **c** A graph comparing IL4, 10, 12B, and 17A levels in patients' subgroups and controls. All parameters represented as mean.

Genotypes of IL17A (−197G/A) polymorphism and its effect on IgE and IL17A serum levels

There was a statistically non-significant difference between IL-17A gene polymorphism and IgE and IL-17A serum levels as demonstrated in Table 9.

Discussion

Allergy is an inflammatory and immune disorder caused by aberrant T helper-2 (Th2) immune reactions caused by excessive exogenous stimulation triggering the IgE receptor to secrete pro-inflammatory cytokines [18]. The differentiation of CD4+ T cells into effectors generating Th2 cytokine profiles, such as IL-4, IL-5, IL-9, and IL-13, is the initial event and the major factor in the development of atopic diseases [27, 28].

In the present study, we found that the peripheral blood eosinophil count was significantly higher in patients with atopy than in the control group. Studies

Table 4 Comparison between dust mites' allergic patients and controls regarding laboratory data (IgE levels, eosinophilic count, and interleukins levels)

The studied parameter	Healthy controls (*N* = 60)	Allergic patients (*N* = 60)	*p* value
[1]PBEC (cells/mm^3)	142.9 ± 31.0	576.9 ± 60.03	< 0.001**
Serum IgE level (IU/ml)	15.5 ± 15.9	276.9 ± 141.2	< 0.001**
IL-4 (pg/ml)	6.72 ± 0.47	44.76 ± 6.3	< 0.001**
IL-10 (pg/ml)	3.5 ± 0.45	2.8 ± 1.2	> 0.05*
IL-12B (pg/ml)	264.46 ± 31.35	170.49 ± 50.72	< .001**
IL-17A (pg/ml)	3.31 ± 0.85	32.2 ± 9.2	< .001**

[1]*PBEC* peripheral blood eosinophil cell count. Student's *t* test was used. All parameters represented as mean and SD. *p value ≤ 0.05 significant while **p value ≤ 0.01 highly significant and* $p > 0.05$ non-significant

Table 5 Comparison between the studied subgroups regarding laboratory data (IgE levels, eosinophilic count, and interleukins levels)

The studied parameter	controls *N* = 60	Asthma *N* = 20	AR *N* = 20	Dermatitis *N* = 20	ANOVA	*p value
PBEC (cells/mm^3)	142.9 ± 31.0	636.3 ± 106.4	576.9 ± 47.8	517.7 ± 25.9	129.888	< .001**
Serum IgE level (IU/ml)	15.5 ± 15.9	342.0 ± 193.0	226.9 ± 82.1	262.0 ± 148.7	11.699	< .001**
IL-4 (pg/ml)	6.7 ± 0.5	51.3 ± 4.0	41.5 ± 4.3	41.5 ± 4.3	290.428	< .001**
IL-10 (pg/ml)	3.5 ± 0.4	2.9 ± 1.3	2.9 ± 1.0	2.6 ± 1.3	1.280	0.296
IL-12B (pg/ml)	264.5 ± 31.3	144.1 ± 28.9	144.1 ± 28.9	223.3 ± 44.5	31.127	< .001**
IL-17A (pg/ml)	3.3 ± .9	42.2 ± 4.1	32.2 ± 4.1	22.2 ± 4.1	218.483	< .001**

PBEC peripheral blood eosinophil cell count. All parameters represented as mean and SD. *p value ≤ 0.05 significant while **p value ≤ 0.01 highly significant and $p > 0.05$ non-significant

on atopic patients in different areas have shown similar results [6, 29].

We also observed that total IgE was higher in atopic patients than in controls. Similar results have been reported in many studies with allergic patients [6, 30–33].

According to measured interleukins levels in our study, the serum level of IL-4 in dust mites allergic patients was significantly higher relative to the control group. IL-4 has been identified as the most important cytokine implicated in the upregulation of in vivo IgE production related to the intensity of eosinophilic inflammation in allergic populations [34]. Elevation of serum IL-4 in allergy was observed in allergic patients and indicated involvement in the allergy pathogenesis [27]. Similar findings have been obtained in other studies in asthma and other atopic diseases [35, 36].

In this study, there was no significant difference in IL-10 serum levels between patients and controls as was previously shown in a study with atopic asthma children from Saudi Arabia [24]. Etiology of allergic inflammation in tissues has been related to increased IL-4 and decreased IL-10 levels in the blood [37]. On the other hand, the increased expression of IL-10 was documented in allergy [38, 39]. Such conflicting results can be explained by the fact that the role of IL10 relates not only to its level but also to other factors including its binding affinity to its receptors. IL-10 may have a positive or negative effect on IgE either by triggering IgE release from plasma cells or by suppressing IgE isotype switches by inhibiting IL-4 and IL-13 [40]. Therefore, IL-10 can have various results, depending on the timing and source. If present early in the immune response, IL-10

Table 6 Genotype and allele frequencies of IL12B (1188 A/C) and IL17A (−197G/A) polymorphisms in patients and controls

The studied parameter		Controls *N* = 60 (%)	Patients *N* = 60 (%)	Fisher exact test	*p* value
IL-12B (+1188 A/C)	**Genotype**				
	AA	54(90.0)	18 (30)	14.344	.008**
	AC	6 (10.0)	40 (66.7)		
	CC	0 (0.0)	2 (3.3)		
	Allele				
	A	114 (95.0)	76 (63.3)	11.574	.010**
	C	6 (5.0)	44 (36.7)		
IL17A (−197G/A)	**Genotype**				
	GG	48(80.0)	16 (26.7)		
	GA	6(10.0)	38 (63.3)	12.192	.029*
	AA	6(10.0)	6 (10.0)		
	Allele				
	G	102 (85.0)	70 (58.3)	16.4	.005**
	A	18 (15.0)	50 (41.7)		

Polymorphism is represented as number and percent. *p value ≤ 0.05 significant while **p value ≤ 0.01 highly significant

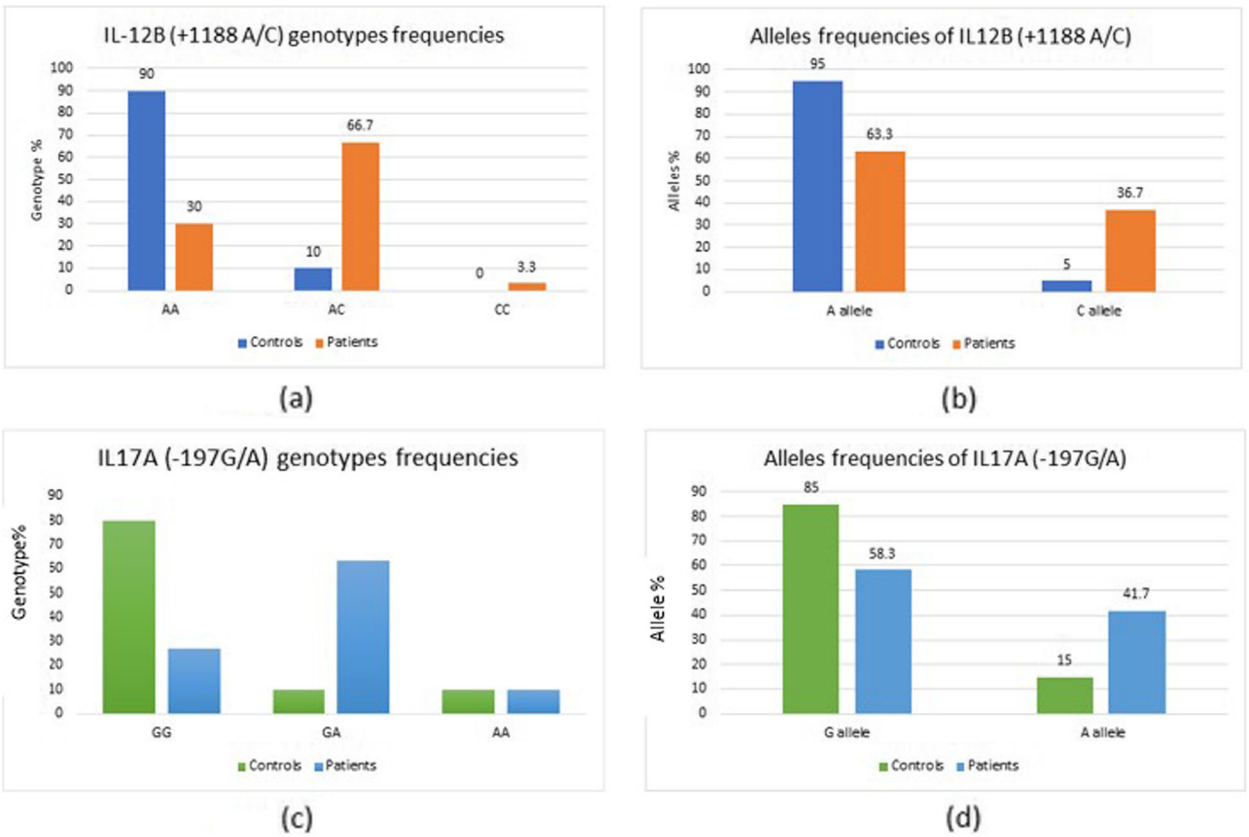

Fig. 5 **a**, **b** Genotypes and alleles frequencies of IL12B (1188 A/C) in patients and controls. **c**, **d** Genotypes and alleles frequencies of IL17A (−197G/A) in patients and controls. Polymorphisms are represented as a percent

should induce allergen non-responsiveness and prevent IgE isotype switching, whereas if present late in the allergic response, IgE secretion by B lymphocytes may be enhanced [41].

IL-12 has multiple biological activities, and it is a key factor that drives Th1 responses and IFN-γ production [42]. Our results demonstrated a significantly lower serum level of IL12B in patients with dust mites allergy as compared to controls as shown in several studies where IL-12 mRNA expression and IL12b levels were lower in atopic patients compared to controls [43–45].

In this study, increased IL-17A levels were detected higher in the serum of dust mites allergic patients as compared to controls. As shown in studies with allergic patients [46, 47]. IL17A works on epithelial cells and other types of inflammatory cells to enhance the secretion of pro-inflammatory cytokines and stimulate neutrophil recruitment [46]. Upregulation of IL-17A in plasma allergic patients can be partly related to increased production of circulating Th17 cells, which are the primary source of IL-17 in vivo [48]. Several studies have shown that IL-17A has been locally increased in bronchial biopsies, bronchoalveolar lavage fluid, and sputum samples from asthmatic patients compared to controls [49, 50] and raised in IL17A in plasma of allergic asthmatics than in controls [51].

Recent studies have suggested that certain molecules' SNPs affect their level of expression or function [52]. Numerous molecules, including cytokines, are now believed to be associated with the pathogenesis of immune-mediated diseases, and some of these molecules' gene polymorphisms are considered candidates as genetic factors in these diseases. In this study, we aimed to investigate the possible association between the interleukin 12B polymorphism rs3212227 and IL17A polymorphism rs2275913 with dust mites' allergic patients.

Our results showed that the frequencies of IL12B A/C and C/C genotypes were significantly higher in patients compared with the control group (p value ≤ 0.01). AA genotype tended to be decreased in allergic patients compared with controls, and the reverse was true in CC genotype. We demonstrated that the A allele was decreased in allergic patients, compared with control with a significant difference. These results indicate that the

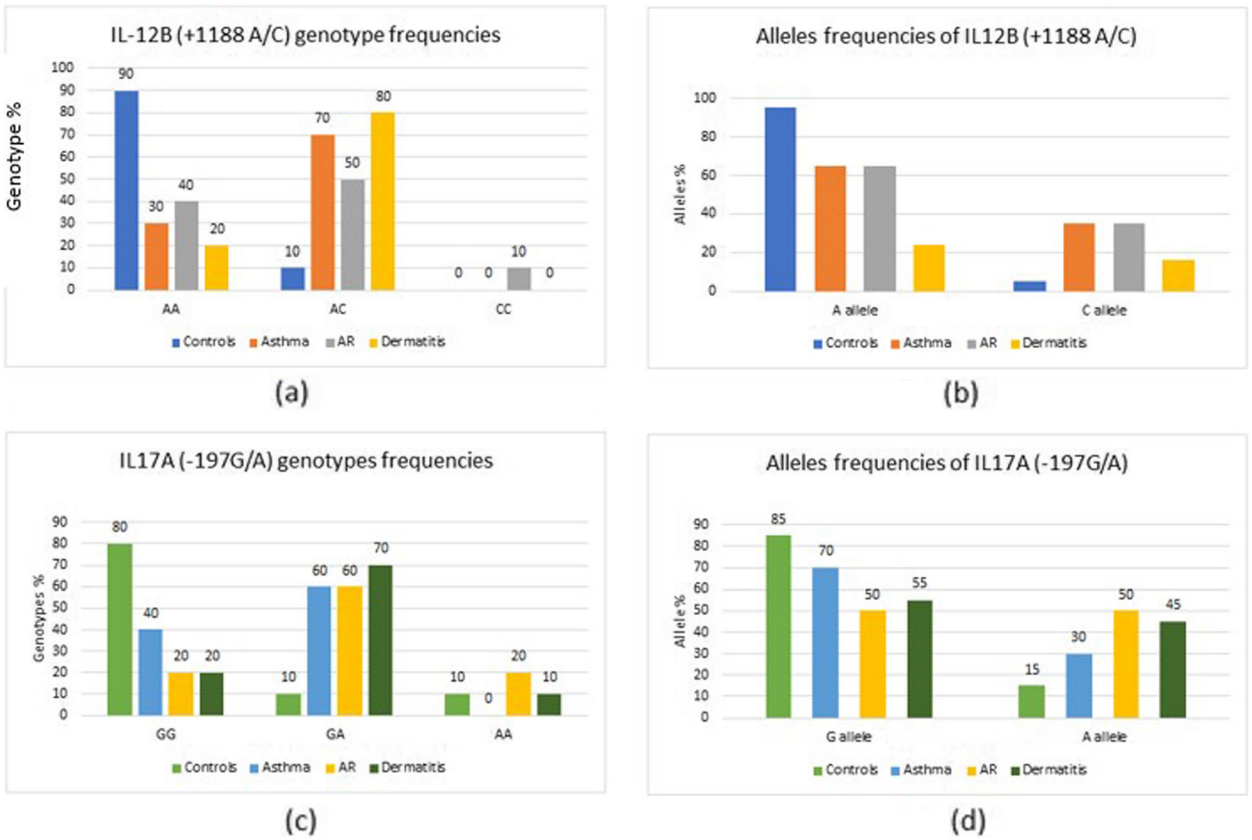

Fig. 6 **a**, **b** Genotypes and alleles frequencies of IL12B (+1188 A/C) in patients' subgroups and controls. **c**, **d** Genotypes and alleles frequencies of IL17A (−197G/A) in patients' subgroups and controls. Polymorphisms are represented as a percent.

IL-12 p40 3′UTR +1188 A/C polymorphism is associated with susceptibility to dust mites allergy in Egyptian patients as presented in a study where the A allele was decreased in atopic dermatitis [8] and other studies showed an increase in C alleles among asthmatic patients [53] and allergic rhinitis [54].

The CC genotype carriers were significantly overrepresented among autoimmune diseases as in ulcerative colitis, Crohn's disease [52], and lepromatous leprosy patients [55].

Our results demonstrated that dust mites' allergic patients presented with higher frequencies of GA genotype in rs2275913 of IL-17A than healthy controls. These results are the same as several studies in asthmatic patients [53–56]. Also, previous researches demonstrated the role of IL17A (rs2275913) gene polymorphism in inflammatory and autoimmune diseases as rheumatoid arthritis [17] and ulcerative colitis [57].

In this study, there was no association between genotypes of the studied SNPs of IL12B and IL17A with their serum levels, as was reported by many studies that found no evidence of polymorphism effect on cytokine expression [55, 58]. However, our findings need to be interpreted with caution as it needs further prospective studies with larger sample sizes and diverse populations to confirm the relationship between IL12B and IL17A SNPs and allergy development.

Conclusion

To our knowledge, this research has for the first time identified a series of Th1/Th2 cytokine and +1188A/C IL12B and −197G/A IL17A SNPs associated with allergy to dust mites in Egyptian patients. IL-12B gene polymorphism at +1188 A/C and IL17A at -197 G/A may be contributing factors in the susceptibility of dust mites' allergy among patients. We found higher frequencies of IL12B rs3212227 AC and CC genotypes in patients compared with the control group (p value ≤ 0.01) and higher frequencies of GA genotype in rs2275913 of IL-17A than controls. These findings highlight the potential genetic influence on allergy development, and it may be possible to predict a particular individual's susceptibility to HDM allergy and the development of new therapeutic strategies.

Table 7 Genotype and allele frequencies of IL12B (+1188 A/C) and IL17A (−197G/A) polymorphisms among studied groups

The studied parameter		Controls *N* = 60 (%)	Asthma *N* = 20 (%)	AR *N* = 20 (%)	Dermatitis *N* = 20 (%)
IL12B (+1188 A/C)	**Genotype**				
	AA	54 (90.0)	6 (30.0)	8 (40.0)	4(20.0)
	AC	6 (10.0)	14 (70.0)	10 (50.0)	16 (80.0)
	CC	0 (0.0)	0 (0.0)	2 (10 .0)	0 (0.0)
	p value		.020*	< .001**	.005**
	Allele				
	A	114 (95.0)	26 (65.0)	26 (65.0)	24 (60.0)
	C	6 (5.0)	14 (35.0)	14 (35.0)	16 (40.0)
	p value		.043*	.043*	.019*
IL17 (−197G/A)	**Genotyping**				
	GG	48(80.0)	8 (40.0)	4 (20.0)	4(20.0)
	GA	6 (10.0)	12(60.0)	12(60.0)	14(70.0)
	AA	6 (10.0)	0 (0.0)	4(20.0)	2(10.0)
	p value		.057	< .001**	< .001**
	Allele				
	G	102 (85.0)	28 (70.0)	20 (50.0)	22 (55.0)
	A	18 (15.0)	12 (30.0)	20 (50.0)	18 (45.0)
	p value		.450	.018*	.038*

Fisher exact test was used. All parameters represented as number and percent. *p value ≤ 0.05 significant while **p value ≤ 0.01 highly significant and $p > 0.05$ non-significant

Table 8 Effect of IL-12B gene polymorphism in allergic patients on IgE and IL-12B serum levels in dust mites' allergic patient

The studied parameter	AA (*N* = 18)	AC (*N* = 40)	CC (*N* = 2)	ANOVA	*p* value
Serum IgE level (IU/ml)	250.3 ± 214.5	285.3 ± 122.1	310.0 ± 0	.272	.764
Serum IL-12b (pg/ml)	164.3 ± 50.5	175.8 ± 51.7	159.8 ± 0	.661	.525

All parameters represented as mean and SD

Table 9 Effect of IL-17A gene polymorphism on IgE and IL-17A serum levels in domestic mites' allergic patient

The studied parameter	GG (*N* = 16)	GA (*N* = 38)	AA (*N* = 6)	ANOVA	*p* value
Serum IgE level (IU/ml)	272.5 ± 226.7	272.6 ± 124.3	290.7 ± 104.1	.108	.898
Serum IL-17A (pg/ml)	34.1 ± 10.4	31.4 ± 9.4	32.3 ± 5.3	.222	.803

All paramete represented as mean and SD

Abbreviations

HDM: House dust mites; IL: Interleukin; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphisms; SNP: Single-nucleotide polymorphisms

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Authors' contributions

NE and AA were involved in protocol development, researched the literature with EE, and conceived the study and laboratory work, then helped and revised the statistical work and results, and wrote the first draft of the manuscript. AA and AK conceived the study and were involved in the laboratory work and revised the statistical work and results. DH and AE helped in gaining ethical approval and patient recruitment. All of the authors help in data analysis. All authors reviewed, edited the manuscript, and approved the final version of this manuscript.

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Availability of data and materials

The authors confirm that data supporting the findings of this study are available within the study.

Ethics approval and consent to participate

The study was been done according to the regulations of the Egyptian Ministry of Higher Education and after having the required approval from the ethical committee of the Faculty of Medicine Ain Shams University (No. FWA 000017585). Written informed consent was obtained from all participants. Patients found allergic to dust mites were referred for immunotherapy whenever accepted by patients.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interest.

Author details
[1]Medical Parasitology Department, Ain-Shams University, Ramsis Street, Abbassia Square, Cairo, Egypt. [2]Internal Medicine, Allergy and Clinical Immunology Department, Faculty of Medicine, Ain-Shams University, Ramsis Street, Abbassia Square, Cairo, Egypt.

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