Zedan *et al.*
Egyptian Pediatric Association Gazette (2023) 71:47
https://doi.org/10.1186/s43054-023-00182-x

Egyptian Pediatric Association Gazette

RESEARCH

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Prevalence of bronchial asthma and correlation between the chemokine receptor 3 gene polymorphism and clinical asthma phenotypes among Egyptian asthmatic children

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Abstract

Background Asthma is a chronic inflammatory airway disease characterized by episodic reversible airway obstruction that variably presents with cough, wheezing, shortness of breath, or chest tightness. It is a multigenetic disease, where both genetic and environmental factors have significant roles in pathogenesis. As regard asthma pathogenesis, the chemokine/chemokine receptor system is considered a key point of the immune response in most allergic diseases. This study was done to estimate the prevalence of bronchial asthma among school age children and explore the association between the underlying gene polymorphisms in chemokine receptor 3 (CCR3) and symptom-based clinical asthma phenotypes among a cohort of Egyptian children.

Results Prevalence of asthma is increasing (20.6%). There are male asthma predominance. Family history of bronchial asthma and allergic diseases are predominant risk factors for asthma development. Sixty asthmatic cases with different clinical phenotypes were compared to 100 healthy controls, results explored that eosinophilic percent and total serum IgE are significantly higher among asthmatic cases versus controls. There are no significant difference regarding eosinophilic percent, serum IgE, and CCR3 *T51C* gene polymorphism among different clinical asthma phenotypes. There is no significant difference as regards degree of severity of asthma and level of asthma control between CCR3 *T51C* gene polymorphism.

Conclusion We conclude the prevalence of bronchial asthma is increasing. Also, eosinophilic percent and serum IgE are elevated in asthma patients, while CCR3 *T51C* gene polymorphism frequency seemed to be more prevalent among asthmatic subjects however without statistical significance. We recommend a prospective study on larger sample size to validate our results.

Keywords Asthma, Phenotype, Chemokine

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Background

Asthma is a chronic inflammatory airway disease characterized by episodic reversible airway obstruction that variably presents with cough, wheezing, shortness of breath, or chest tightness [1].

Zedan et al. (2009, 2013, and 2014) previously proposed clinical asthma phenotyping based on symptomatology after validation of these symptoms, and they categorized different clinical phenotypes [shortness of breath (SOB), cough, and wheeze] according to their lung functions, specific inflammatory biomarkers, genetic profiles, and their response to asthma medications [2–4].

More recently, investigators though that asthma is a multidimensional disease, where both genetic and environmental factors play significant roles in pathogenesis [5].

A positive family history of asthma and atopy, genetic polymorphisms, and epigenetic markers have all been significantly correlated. In addition to, early onset respiratory tract infections, allergic co-morbidities, and active smoking in adolescence are significant risk factors [6].

The chemokine/chemokine receptor system is considered a key point of the immune response in most allergic diseases [7]. The expression of distinct chemokines within the airway has led to the realization that there might be specific profiles of chemokines that mediate clinical presentations of asthma [8]. The inflammatory chemokines CCL9, CCL10, and CCL11 are predominantly induced by interferon (IFN)-γ and share an exclusive chemokine receptor named chemokine receptor 3 (CCR3) [9]. Numerous studies have reported association between up- or downregulation of one or more CCR3 ligands and a specific disease state.

The aim of this study is to study the specific features of each clinical phenotypes and if there is association between them and the CCR3 *T51C*genepolymorphisms.Also to detect prevalence of asthma and its clinical phenotypes.

Study design and patient recruitment

This study is composed of 2 parts (Fig. 1, 2 and 3):

Cross-sectional study

Including 602 children from Mansoura city primary and preparatory schools with the age ranges from 6 to 16 years old. They were screened for asthma symptoms and predisposing risk factors according to ISSAC questionnaire [10, 11].

Case control study

Case control study comprising 60 asthmatic children aged from 6 to 16 years and 100 age and sex matched controls. Diagnosis of asthma and its level of control was done according to Global Initiative of Asthma (GINA 2020) [12]**.** Asthma patients with comorbidities were excluded. Patients were recruited from outpatient clinics of Allergy and Clinical Immunology unit in Mansoura University Children's Hospitals, and the molecular analysis was done in Clinical Pathology Department Faculty of Medicine at Mansoura University, Egypt.

Study duration

The study was carried out over 3 years (from October 2019 to September 2021).

Selection criteria for the patients

Inclusion criteria

- Asthmatic children aged 6 to 16 years old diagnosed according to presence of typical asthma symptoms (according to the recent established guidelines of global initiative for asthma management and prevention in 2019). Confirmed variable expiratory airflow obstruction as evidenced by improvement in prebronchodilator FEV1 of > 12% predicted after salbutamol (200ug) (GINA, 2019) [13].
- After validation of asthma symptoms, the asthmatic children were subdivided into three clinical phenotypes according to symptoms, the 1st group was cough predominant asthma phenotype, 2nd group was cough with allergic rhinitis (AR) phenotype and 3rd group was cough and wheeze asthma phenotype. In cough-predominant asthma cough is the most predominant symptom but other symptoms are also present [14]. Wheezes were defined by the patient as creaking, rattle, whistling, and jingling [15]. The criterion for defining allergic rhinitis was the combination of history, allergy diagnosis such as total serum IgE to confirm the correlation between the characteristic symptoms, such as rhinorrhea, nasal obstruction, sneezing, nasal itch, and potential allergens [16].

Exclusion criteria

- Asthmatic patients with comorbidities such as cardiovascular diseases, chronic pulmonary disease and inflammatory diseases that influence cytokine profile and chemokine network as rheumatoid arthritis and inflammatory bowel diseases [17].
- Patients under immunotherapy that alter cytokine profile [18].

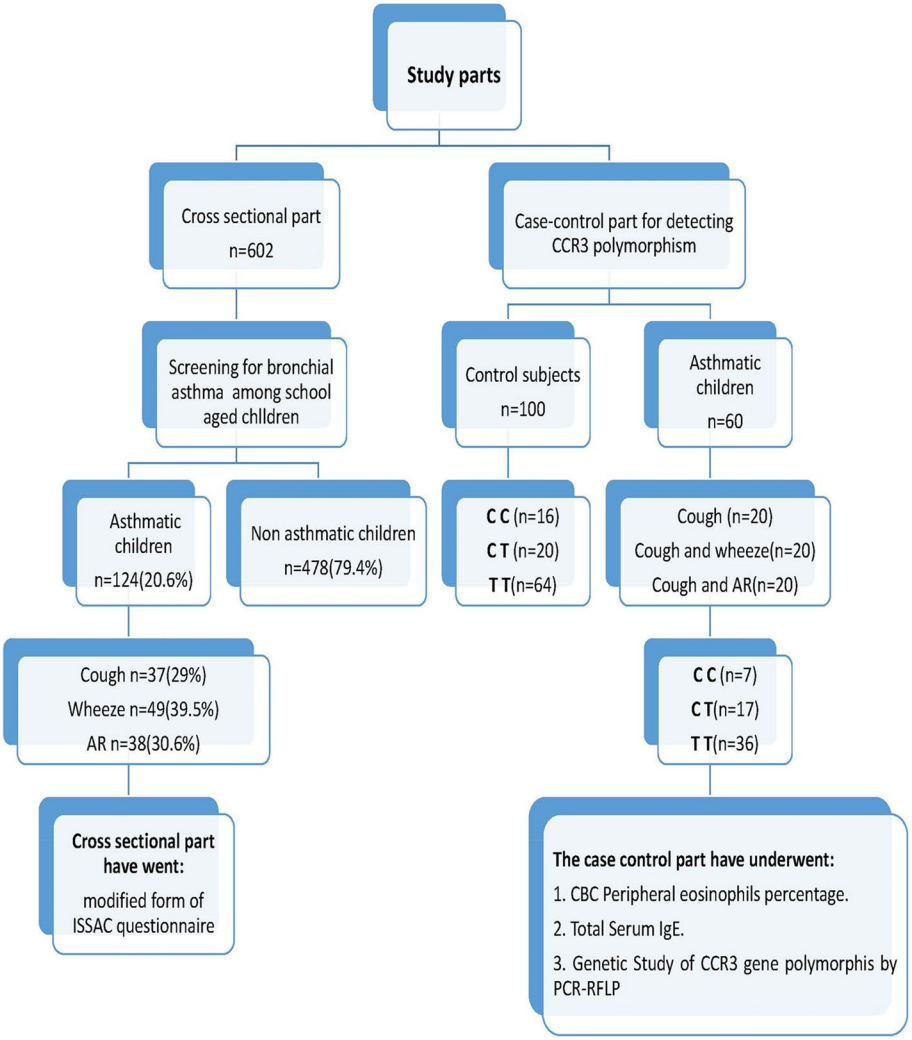

Fig. 1 Flow chart of the study design and included subjects

Controls

Healthy children of matched age and sex without apparent evidence of personal and family history of allergic diseases have been enrolled in the study.

Methods

Study participants underwent the following:

Cross-sectional study

A. ***Sample size***

Sample size of cross sectional was calculated based on the result of a study done in 2009 that estimated the overall prevalence of childhood asthma was 7.7% in the Nile Delta region of Egypt [15]. Sample size is calculated according to the following equation [19].

$$n = Z^2P(1-P)/d^2; n = 1.96 \times 1.96 \times 0.77 \times 0.23/0.0025 = 272$$

where n = sample size, Z = Z statistic for the level of confidence of 95%, ($Z = 1.96$), P = expected prevalence (proportion), ($P = 23\%$), d = precision, ($d = 0.05$).

Thus, $n = 272 \times 2$(design effect) = 544 and adding 10% nonresponse rate, the sample would be 600.

B. ***Sampling technique***

A random sample of school aged children aged from 6 to 16 years old was obtained using an online form of the questionnaire instead of usual written form due to corona virus pandemic.

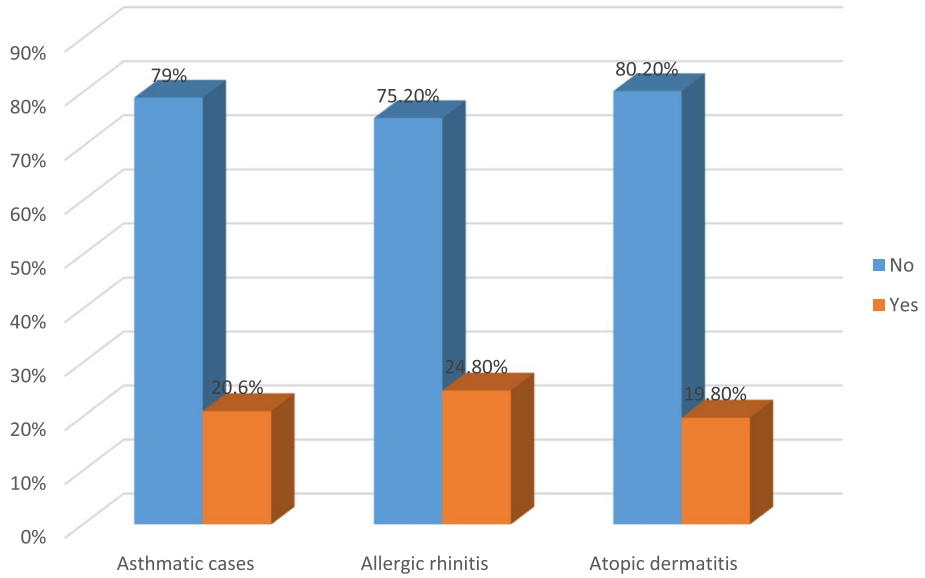

Fig. 2 Prevalence of asthma, allergic rhinitis, and atopic dermatitis among the studied group

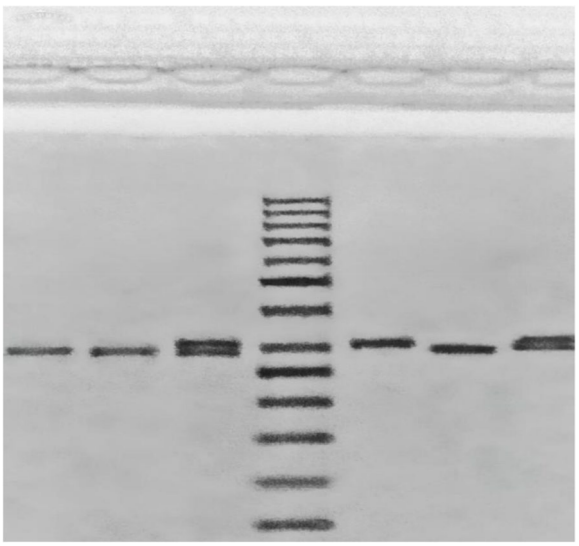

Fig. 3 CCR3T51C gene polymorphism and genotypes showing from right to left lane 1, 5 represent CT genotype, lane 2, 6, and 7 represent TT genotype and uncut lane 3 represents CC genotype

3. *Methodology*

Study instrument and operational definitions: modified form of the International Study of Asthma and Allergy in Children questionnaire (ISAAC) (ISAAC, 1998; ISAAC, 2000). All questions will be translated from English to Arabic and their syntax will be verified by back-translation.

The questionnaire included questions about established respiratory and allergic diagnoses, respiratory and allergic symptoms in the past 12 months, predominant clinical asthma symptoms, family history of respiratory and allergic disorders, and socio-economic status. Location of dwelling will be classified as urban or rural based on whether the child lived in the city or the surrounding agricultural area. Potential correlates of respiratory outcomes will be assessed including parental asthma, allergic rhinitis, eczema, current smoking and highest level of education for each of the mother and the father. Additional risk factors will include smoking during pregnancy, passive smoking during pregnancy, the presence of dog and cat in the home, and dampness at home. Evaluation of health care utilization and morbidity will be based on answers to questions regarding medication use for breathing, specialist or general practitioner visits for breathing conditions.

Case control study

1. ***History taking and examination***

Full clinical history taking and examination with validation of asthma symptoms. The patient's data were registered including: age, sex, residence, nutritional history, parental consanguinity, family history of asthma, clinical asthma phenotypes, exposure to triggers, and dietetic history.

2. ***Laboratory testing***

Blood samples were taken for complete blood count for eosinophilic count and percent using automated cell

counter, total serum Ig E using ELISA technique and PCR–RFLP was performed for detection of CCR3 T51C gene polymorphism.

For genotype study, blood samples were collected in EDTA tubes and used for DNA extraction using micro centrifuged columns then amplified by PCR. DNA extraction kits (QIAamp, QIAGEN Inc., Germany, Cat No. 51104) were used**.** PCR process using sense primer 5′-CTTTGGTACCACATCCTACCA-3′ and the antisense primer 5′-TGAGAGGAGCTTACACATGC-3′. PCR was performed using Taq polymerase and an attached buffer (including MgCl2 at the final concentration of 1.5 mM) as follows: initial denaturation 95 ℃ for 5 min, 45 cycles of denaturation for 30 s and final extension at 72 ℃ for 10 min [20]. This was followed by digestion by restriction enzyme N1a III then gel electrophoresis on 2% agarose to detect CCR3 *T51C* gene polymorphism.

Statistical analysis

The collected data were coded, processed, and analyzed using IBM SPSS program (Version 22.0) (Social package for statistical sciences, IBM Corporation; Armonk, NY, USA). The Hardy–Weinberg equilibrium was tested by the Chi-squared test for the frequencies of the CCR3 genotypes. Qualitative data were described using number and percent. Quantitative data were described as range (minimum and maximum) or mean (standard deviation) according to data normality. *P* values less than or equal 0.05 (5%) was considered statistically significant. The tests used to analyze categorical data were chi-square test, Fisher's exact or Monte Carlo. Tests used in analysis of quantitative data were Mann–Whitney test, Student's *t* test, Kruskal–Wallis test.

Odds ratio (OR) and 95% confidence intervals (CI) were calculated from the β-coefficients and their standard errors.

Results

The prevalence of asthmatic children was 20.6% while allergic rhinitis 24.8% and atopic dermatitis was found in 19.8%. (Table 1).

Asthmatic males were significantly more than asthmatic females (26.5% vs 16.1%) ($p=0.002$). Also, asthmatic children who were on mechanical ventilation during neonatal period were statistically more than those who did not had mechanical ventilation (36.1% vs 19.6%, *p* value 0.01). Also, children who were breast fed > 1 year developed bronchial asthma were statistically less than those breast fed < 1 year (*p* value 0.01) while there was no significant statistical difference as regards other demographic factors as age, residence, crowding index, birth weight, NICU admission, breast feeding, routine vaccination, and optional vaccination (flu/pneumonia) ($p>0.05$) (Table 2).

Table 1 Prevalence of atopic diseases among studied group in El-Mansoura City 2019–2021

Risk factor	Total *n* = 602
Asthmatic cases	
Yes	124 (20.6%)
No	478 (79%)
Allergic rhinitis	
Yes	149 (24.8%)
No	453 (75.2%)
Atopic dermatitis	
Yes	119 (19.8%)
No	483 (80.2%)

This table showed that prevalence of asthmatic children was 20.6% while allergic rhinitis 24.8% and atopic dermatitis was found in 19.8%

Asthmatic children who were not previously exposed to parasitic infection are statistically more than children who were previously exposed to the same infection (*P* value = 0.03). Children with history allergic rhinitis develop asthma significantly more than those who didn't have history of allergic rhinitis (*p* value = 0.0008). Also children with family history of allergic disease and bronchial asthma develop asthma significantly more than those without family history of allergic disease and bronchial asthma (*p* value = 0.0003, 0.0001, 0.02, and 0.05) respectively while there were no significant difference as regards parents' history of hay fever and atopic dermatitis ($p<0.05$) (Table 3).

There were statistically significant differences as regards numbers of males in cough asthma and cough with allergic rhinitis phenotypes when they were compared to females (14 vs 6, 16 vs 4. *P* value 0.02) respectively, While cough with wheeze asthma phenotype shows female predominance. However, higher percent of patients having cough asthma phenotype without history of atopic dermatitis as compared to other asthma phenotypes (cough with allergic rhinitis and cough and wheeze phenotypes) (85% vs 55% and 50%. *P* value 0.04) respectively. No significant differences were noted between clinical asthma phenotypes regarding: age, residence, crowding index, smoking exposure, consanguinity, and type of feeding (Table 4).

Both eosinophilic percent and total IgE were significantly higher among asthmatic cases when compared to control group (*P* value = 0.009, < 0.001) respectively (Table 5).

The eosinophilic percent was higher in cough and wheeze phenotype when compared with other

Table 2 Epidemiologic features of asthmatic children

Variable	Total *n* = 602	Asthmatic *n*=124 (%)	Significance test^{X2}	*P* value	OR (95% CI)
Age groups					
5–10 years	252	60 (23.8)	2.5	0.1	1.3
> 10–16 years (*r*)	346	64 (18.5)			(0.9–2)
Sex					
Male	260	69 (26.5)	9.8	0.002*	1.8
Female (*r*)	342	55 (16.1)			(1.2–2.8)
Residence					
Urban	226	53 (23.5)	1.8	0.5	1.3 (0.8–2)
Suburbs with lots of gardens	18	4 (22.2)	0.1		1.2 (0.3–3.9)
Suburbs with few of gardens	37	7 (48.9)	0.001		1.01 (0.4–2.4)
Rural (*r*)	321	60 (18.7)			
Crowding Index					
≤ 2 persons/room (*r*)	530	113 (21.3)	1.4	0.2	
> 2 persons/room	72	11 (15.3)			0.6 (0.3–1.3)
Birth weight in kg #					
Normal (> 2.5 kg) (*r*)	550	111 (20.2)	0.6	0.4	1.3
LBW (< 2.5 kg)	52	13 (25)			(0.6–2.5)
NICU admission					
Yes	61	15 (24.6)	0.6	0.4	1.2
No(*r*)	541	109 (20.1)			(0.6–2.4)
Mech. ventilation					
Yes	36	13 (36.1)	5.6	0.01*	2.3
No(*r*)	566	111(19.6)			(1.1–4.7)
Breast feeding					
Yes (*r*)	519	102 (19.7)		0.15	1.4
No	83	22 (26.5)	2.05		(0.8–2.5)
Duration of breast feeding #					
> 1 year(*r*)	498	91 (18.3)	5.09	0.01*	
6 months–1 year	47	15 (31.9)	5.7		2.09 (1.09–4.03)
< 6 months	57	18 (31.6)			2.06 (1.113.7)
Routine vaccination					
Yes(*r*)	589	122 (98.4)	0.2	0.6	
No	13	2 (1.6)			0.6 (0.1–3.1)
Optional (flu and/or pneumonia vaccine)					
Yes (*r*)	258	56 (45.2)	0.3	0.5	
No	344	68 (54.8)			0.8 (0.51.3)

Row percent is considered, *(r)* reference group, *Significance test* χ2 chi-square test, *OR* odd's ratio, *CI* confidence interval and *significant *p* value < 0.05

The above table showed that the asthmatic males were significantly more than asthmatic females (26.5% vs 16.1%) (*p* value = 0.002) Also, there were a significant difference as regards mechanical ventilation during neonatal period and breastfeeding duration (*p* value = 0.01). While there were no significant statistical difference as regards other demographic factors as age, residence, crowding index, birth weight, NICU admission, breast feeding, routine vaccination, and optional vaccination (flu/pneumonia) (*p* > 0.05)

phenotypes without significant difference. Also, total IgE was higher in cough phenotype when compared to other phenotypes without significant difference (Table 6).

Also, there was higher association of CCR3 *T51C* among studied asthmatic phenotypes as regards TT than CT without statistically significant difference (*P* value > 0.05) (Table 7).

No difference between CCR3 *T51C* gene polymorphism (CC, CT, TT) regarding either asthma severity or level of asthma control (Table 8).

Discussion

Asthma is not just one disease; it is characterized by overlapping physiological and clinical features. Its heterogenicity can be subclassified into a diverse array of

Table 3 Associated medical risk factors for development of childhood bronchial asthma among studied children

Risk factor	Total *n* = 602	Asthmatic *n* = 124(%)	Significance test χ2	*P* value	OR (95%CI)
Past history of infection:					
Measles	24	2 (8)	3.1	0.07	0.2(0.06–1.2)
Whooping cough	52	9 (17)	1.1	0.2	0.6(0.3–1.4)
TB	7	1 (14)	0.4	0.5	0.5(0.06–4.4)
Parasites	154	24 (15)	4.6	0.03*	0.5(0.3–0.9)
None (*r*)	365	88 (24)			
Allergic rhinitis					
Yes	149	45 (30)	11.15	.0008*	2 (1.3–3.1)
No(r)	453	79 (17.4)			
Atopic dermatitis					
Yes	119	27 (22.7)	0.39	0.5	1.16 (0.7–1.8)
No(*r*)	483	97 (20)			
Mother history of allergic disease					
Yes	119	41 (34.5)	17.4	0.0003*	2.5 (1.6–3.9)
No	483	83 (17.2)			
Mother history of bronchial asthma					
Yes	64	25 (39.1)	14.9	0.0001*	2.8 (1.6–4.9)
No	538	99 (18.4)			
Mother history of hay fever					
Yes	14	5 (35.7)	1.99	0.15	2.1 (0.7–6.6)
No	588	119 (20.2)			
Mother history of atopic dermatitis					
Yes	41	11 (26.8)	1.04	0.3	1.4 (0.7–2.9)
No	561	113 (20.1)			
Father history of allergic diseases					
Yes	76	23 (30.3)	4.9	0.02*	1.8 (1.06–3.1)
No	526	101(19.2)			
Father history of bronchial asthma					
Yes	52	16 (30.8)	3.6	0.05*	1.8(0.9–3.9)
No	550	108 (19.6)			
Father history of hay fever					
Yes	3	0 (0)	0.78	0.3	0.7(0.7–0.8)
No	599	124 (20.7)			
Father history of atopic dermatitis					
Yes	21	7 (33.3)	2.2	0.1	2.0 (0.7–5.06)
No	580	116 (20)			

Row percent is considered, (*r*): reference group, *Significance test* χ2 chi-square test, *OR* odd's ratio, *CI* confidence interval and *significant *p* value < 0.05

The above table showed the asthmatic children who were not previously exposed to parasitic infection are statistically more than children who were previously exposed to the same infection (*P* value = 0.03). Children with history allergic rhinitis develop asthma significantly more than those who did not have history of allergic rhinitis (*p* value = 0.0008). Also, children with family history of allergic disease and bronchial asthma develop asthma significantly more than those without family history of allergic disease and bronchial asthma (*p* value = 0.0003, 0.0001, 0.02, and 0.05) respectively while there were no significant difference as regards parents' history of hay fever and atopic dermatitis ($p < 0.05$)

phenotypes and endotypes [21]. Identifying true endotypes of asthma and their underlying mechanisms is a prerequisite for achieving better mechanism-based treatment targets, and ultimately delivery of genuinely stratified medicine in asthma [22].

The aim of this study is to study the specific features of each clinical phenotypes and if there is association between them and the CCR3 *T51C* gene polymorphisms. Also, to detect prevalence of asthma and its clinical phenotypes.

Table 4 Demographic and clinical features of different clinical asthma phenotypes

Asthma phenotypes					
	Cough asthma phenotype *n* = 20	**Cough with allergic rhinitis phenotype *n* = 20**	**Cough and Wheeze phenotype *n* = 20**	**Test of significance**	***P* value**
Age in years mean ± SD	9 ± 3.5	9.3 ± 3.4	8.9 ± 3.2	0.06	0.9
Duration of asthma in years	5.5 (1–11)	5.5 (2–12)	5.5 (3–13)	1.06	0.5
Gender no. %					
Male	14 (70)	16 (80)	8 (40)	$\chi^2 = 7.4$	0.02*
Female	6 (30)	4 (20)	12 (60)		
Residence no. %					
Rural	14 (70)	8 (40)	10 (50)	$\chi^2 = 3.7$	0.1
Urban	6 (30)	12 (60)	10 (50)		
Crowding Index no. %					
≤ 2 persons/room	13 (65)	13 (65)	14 (70)	$\chi^2 = 0.1$	0.9
> 2 persons/room	7 (35)	7 (35)	6 (30)		
Family history of allergic diseases no. %					
Yes	5 (25)	10 (50)	7 (35)	$\chi^2 = 2.7$	0.2
No	15 (75)	10 (50)	13 (65)		
History of allergic rhinitis no. %					
Yes	4 (20)	20 (100)	8 (40)	$\chi^2 = 27.8$	> 0.001*
No	16 (80)	0 (0)	12 (60)		
History of atopic dermatitis no. %					
Yes	3 (15)	9 (45)	10 (50)	$\chi^2 = 6.17$	0.04*
No	17 (85)	11 (55)	10 (50)		
Family history of smoking no. %					
Yes	8 (40)	7 (35)	12 (60)	$\chi^2 = 2.8$	0.2
No	12 (60)	13 (65)	8 (40)		
Parental consanguinity no.%					
Yes	4 (20)	4 (20)	5 (25)	$\chi^2 = 0.19$	0.9
No	16 (80)	16 (80)	15 (75)		
Type of feeding during infancy no. %					
Breast feeding	11 (55)	10 (50)	11 (55.5)	$\chi^2 = 5.7$	0.2
Formula feeding	2 (10)	0 (0)	4 (20)		
Mixed	7 (35)	10 (50)	5 (25)		

* *P* value less than 0.05 is considered statistically significant

* χ^2 chi-square test

Cough phenotype and cough with allergic rhinitis phenotype showed a significant male predominance, while cough and wheeze phenotype showed a significant female predominance (*p* value = 0.02). History of allergic rhinitis was significantly higher in cough and allergic rhinitis phenotype (*p* value = 0.001). Cough asthma phenotype significantly were not have history of atopic dermatitis when compared with others phenotypes (*p* value = 0.04)

Table 5 Airway inflammatory biomarkers characteristics among the studied cases and control

Variables	**Cases *n* = 60**	**Control *n* = 100**	**Test of significance**	***P* value**
Eosinophilic percentage (%)				
Median(minimum–maximum)	4.0 (0.10–46.4)	2.9 (0.15–10.6)	$Z = 2.5$	0.009*
Total IgE (IU/ml)				
Median(minimum–maximum)	45.6 (0–1143)	5.9 (2.8–45.6)	$Z = 6.3$	< 0.001*

Test of significance: Mann–Whitney test

* Statistical significance was defined as $P < 0.05$

The above table showed that the eosinophilic percent and total IgE were significantly higher among asthmatic cases when compared to control group (*P* value = 0.009, < 0.001) respectively

Table 6 Airway inflammatory biomarkers characteristics of the different clinical asthma phenotype

Variables	Cough ($n=20$)	Cough with allergic rhinitis ($n=20$)	Cough and wheeze ($n=20$)	Significance testx^2	*p* value
Eosinophilic percentage (%)					
Median (minimum–maximum)	2.8(0.1–13.2)	4.0(0.5–46.4)	5.1(0.6–16.5)	$\chi^2=3.8$	0.1
Total IgE (IU/ml)					
Median (minimum–maximum)	61.9(0–1013.0)	54.5(0–537.9)	28.7(0.0–1143)	$\chi^2=0.01$	0.9

Test of significance: Kruskal–Wallis test. *r* reference group

* Statistical significance was defined as $P<0.05$

The above table showed that no significant difference as regards inflammatory biomarkers (eosinophilic percent and total IgE) between different clinical asthma phenotypes

Table 7 Association of CCR3 *T51C* genotypes among the studied asthmatic phenotypes

CCR3 *T51C*	Cough phenotype *N* (%)	Cough with allergic rhinitis phenotype *N* (%)	Cough and wheeze phenotype *N* (%)	OR	CI (95%)	*P* value
TT (mutant type)	13 (65)	13 (65)	10 (50)	$OR_{1,2}=0.75$ $OR_{1,3}=2.6$ $OR_{2,3}=5.2$	$CI_{1,2}=0.30–1.82$ $CI_{1,3}=0.51–5.55$ $CI_{2,3}=0.5–54.0$	$P_{1,2}=0.6$ $P_{1,3}=0.3$ $P_{2,3}=0.1$
CT (heterozygous)	5 (25)	6 (30)	6 (30)	$OR_{1,2}=0.68$ $OR_{1,3}=1.6$ $OR_{2,3}=4.00$	$CI_{1,2}=0.24–1.90$ $CI_{1,3}=0.14–25.2$ $CI_{2,3}=0.3–47.11$	$P_{1,2}=1.0$ $P_{1,3}=1.0$ $P_{2,3}=0.5$
CC (*r*) (wild type)	2 (10)	1 (5)	4 (20)	1	–	–

r reference group

* statistical significance was defined as $P<0.05$

The above table showed that there was higher association of CCR3 *T51C* among studied asthmatic phenotypes as regards TT than CT without statistically significant difference ($P>0.05$)

Table 8 Association between (CCR3 T51C) genotype of asthmatic patients regards asthma severity and level of control

	Genotypes			*P value*
	CC	CT	TT	
	$n=7$	$n=17$	$n=36$	
Degree of asthma severity				
Mild (30)	5 (16.7%)	7 (23.3%)	18 (60%)	0.8
Moderate (24)	2 (8.3%)	8 (33.3%)	14 (58.3%)	
Severe (6)	0 (0%)	2 (33.3%)	4 (66.7%)	
Level of asthma control				
Controlled (37)	6 (16.2%)	8 (21.6%)	23 (62.2%)	0.3
Partially controlled (10)	0 (0%)	5 (50%)	5 (50%)	
Uncontrolled (13)	1 (7.7%)	4 (30.8%)	8 (61.5%)	

Statistical significance was defined as $P<0.05$

* Test of significance: Monte Carlo test

There were no difference in degree of severity of asthma and level of asthma control among different CCR3 *T51C* genotypes

In our study, the prevalence of asthmatic children was (20.6%). This was in agreement with many authors who reported that asthma has increased in the last 30 years [23, 24].

The increase in the prevalence of pediatric asthma may be explained by the increasing exposure to exogenous factors such as outdoor pollutants, for example ozone, sulphur dioxide, and cigarette smoke, a reduction in host resistance, or a combination of both [25].

Few studies evaluated asthma prevalence in Egypt. Khallaf et al. [26] reported that asthma prevalence was 4.8% in Egypt, using a survey including 115 health centers in five governorates provided morbidity figures for acute respiratory infections from 75.789 records of Egyptian infants and children aged less than 4 years.

El-Hefny [27] found that asthma prevalence was 8.2%. Using a questionnaire among 13.028 children 3–15 years old.

In Zedan et al. [15] study estimation of prevalence of questionnaire-diagnosed asthma revealed that the overall prevalence of childhood asthma was 7.7% in the Nile Delta region of Egypt.

This rates differences between all these studies may be due to various different geographical, social, and environmental factors in these localities. Also, it may be due to the varying level of education and medical orientation across parents. Also, variation in the definition of asthma, instrument used to define it, age group taken,

methodology adopted, and urban–rural difference is responsible for this varied observation [28].

Asthma seemed to be predominant in males than females in the current study. Male predominance was explained by the hypothesis that boys have a more severe airway hyper-responsiveness and this may contribute to the higher prevalence of asthma in boys [29].

This finding is consistent with Narayana [30] study reported similar results as dry cough in the past 12 months was significantly higher among the male subjects. Furthermore, the prevalence of nasal symptoms was more among males. In the absence of seasonal rhinitis, 25% males and 10.5% females had nasal symptoms in the form of clear nasal discharge. This difference was statistically significant.

However El-Saify et al. [31] shows increased male susceptibility to asthma till puberty then females tend to be more susceptible in adolescence.

In our study the exposure to mechanical ventilation during the neonatal period was associated with increased incidence of asthma. This may be because of bronchiolar remodeling which was described after mechanical ventilation even for a short period and thus predispose to asthma later on [32]. Furthur more, preterm birth and neonatal factors associated with it may result in lung damage that, later in life, increases the risk for asthma. In Hislop et al. [33] and Hislop and Haworth [34] studies, a permanent impairment of alveolar development and hypertrophy of airway smooth muscle in VLBW infants who were subject to mechanical ventilation was found to induce airway remodeling, which may explain the association between mechanical ventilation during the neonatal period and bronchial hyperresponsiveness at 12 years.

This is in accordance with Mai et al. [35] and Kallen et al. [36]. In which mechanical ventilation during the neonatal period was associated with bronchial hyper responsiveness at age 12 and the risk was high after mechanical ventilation.

In this study results breast fed infants in both studies (case control and cross sectional) seemed less likely to develop childhood asthma later on than those who were artificially fed. This finding was explained by the hypothesis that gut microbiota, in particular, play an important role in training the naïve infant immune system [37]. Human milk contains live microbes that help seed the infant gut, as well as human milk oligosaccharides (HMOs) that provide a selective substrate for gut microbiota [38]. Moreover, direct skin-to-skin contact during breastfeeding may provide an additional source of protective maternal microbes to the nursing infant [39].

In our study, there was a significant association of past history of parasitic infection and allergic rhinitis in asthmatic cases.

Similar to Warrell and colleagues who have suggested that high levels of IgE have been found in children in areas where allergic disease was reported to be rare, reflects an IgE response to parasite infection that may, somehow, inhibit the development of atopy [40].

The protective effects of enteric infections against atopy appear to be greater than for respiratory pathogens. There has been interest also in the potential protective effects against atopy of exposure to mycobacteria. Early observations of an inverse relationship between tuberculin responses and atopic diseases have not been replicated [41].

Children with family history of allergic disease and bronchial asthma were subjected to asthma more than those without family history of allergic disease and bronchial asthma. Asthma develops due to interaction between gene and environment and a parental history of atopy/ asthma is an index of susceptibility to asthma. Maternal influence is probably more than paternal influence, particularly in children less than 5 years of age possibly due to trans-placental transfer of allergens or cytokines to the fetus [42].

In Lawson et al. [43] study in Saskatchewan, Canada, there was a high prevalence of early respiratory illness, related with positive family history of asthma.

In Halim et al. [44] in Ismailia, there is a strong correlation between family history of asthma and prevalence of asthma.

Despite running in families, identification of asthma gene has been elusive with over 100 genes found to be associated with asthma. There is a report of no familial association as well [45].

In this study, the eosinophilic percent and total IgE were significantly higher among asthmatic cases when compared to control group. This reflects the interaction of specific IgE with causally significant allergens on the surface of mast cells and basophils induces the release of preformed and synthesized de novo mediators causing acute inflammation of bronchi accompanied by cell migration in respiratory mucosa and formation of cell infiltrates, including eosinophils, basophils, and Th2-lymphocytes with the involvement of macrophages, monocytes, mast and epithelial cells, platelets, neutrophils, and fibroblasts [46].

This is in agreement with Zedan [47] study, reporting that total serum IgE was significantly increased in asthmatic cases versus controls.

Our results came in accordance with Wang and his colleagues [48], finding that the levels of serum total Ig E were significantly higher in the asthmatic children than in control children.

Also, we could not find significant difference between studied asthmatic phenotype, degree of asthma severity

and level of control with CCR3 *T51C* gene polymorphism. Because asthma is a multifactorial disease, the genetic component may be derived from the combined effect of numerous genes. Individual genes may act independently or in combination with other genes in the same biological pathway, resulting in variable effects [49].

The difference between the different studies could be explained owing to the heterogeneity among the different ethnic populations.

An animal study showed that CCR3 disruption significantly curtails eosinophil recruitment to the lung after allergen challenge [50].

This results may be supported by Ma et al. [51] found that CCR3 − / − mice repeatedly sensitized by epicutaneous application of ovalbumin failed to attract eosinophils to the skin. Therefore, CCR3 is critical for eosinophil recruitment to both the skin and the lung and in the development of airway hyper-responsiveness.

There is no statically significant difference between level of asthma control and degree of asthma severity as different CCR3T51C genotypes.

Similar to Zedan et al. [47] Study, showed no significant difference regarding degree of asthma severity and level of asthma control with CCR3 T51C gene polymorphism.

Conclusion

Our study highlights different asthma phenotypes in relation to CCR3T51C in cohort of Egyptian children, our results suggest that the polymorphisms of CCR3 *T51C* genotype are not associated with asthma susceptibility. The present results must be confirmed with larger-scale studies in the future; however, this could not be generalized because of the different socio-demographic and ethnic aspects. Also, this study explored important protective factors against asthma (e.g., breast feeding) and other risk factors for asthma (e.g., smoking exposure) which may help in ameliorating asthma development.

Abbreviations

AR	Allergic rhinitis
CCR3	Chemokine receptor 3
DNA	Deoxyribonucleic acid
EDTA	Ethylene di-amine tetra-acetic acid
ELISA	Enzyme-linked immuno-sorbent assay
GINA	Global initiative for asthma
HMOs	Human milk oligosaccharides
IFN-γ	Interferon gamma
IgE	Immunoglobulin E
ISSAC	The International Study of Asthma and Allergy in Childhood
NICU	Neonatal intensive care unit
PCR	Polymerase chain reaction
SOB	Shortness of breath

Acknowledgements

The authors would like to thank the patients and their care givers for their cooperation, without their help this work could not be accomplished. Also, they would like to thank pediatric dentistry and nursing staff members of Mansoura University for their efforts.

Authors' contributions

All authors contribute to the study conception and design. HS wrote the first draft of the manuscript and revision of manuscript was done by MMZ (being the major contributor in writing manuscript) and SAEM. EOK contribute to statistical analysis and data interpretation. MEW contribute to material preparation, data collection and analysis. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

No available fund.
All authors have actively participated in all steps of this work, reviewed and agreed upon the manuscript contents.

Availability of data and materials

All the data supported the results can be found within the article.

Declarations

Ethics approval and consent to participate

Informed consents were taken from caregivers of both cases and controls. The Institutional Research Board (IRB) of Faculty of Medicine, Mansoura university, Egypt approved the study on September 2018 with code no: MS.18.09.271.

Consent for publication

A written informed consent for publication was taken from parents of eligible children.

Competing interests

The authors declare that they have no competing interests.

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Received: 10 May 2023 Accepted: 6 June 2023
Published online: 26 September 2023

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