



CD14 tobacco gene–environment interaction in atopic children



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ABSTRACT

Studying gene–environment interactions may elucidate the complex origins of atopic diseases. The aim of this study was to evaluate the association of CD14 polymorphisms and atopy in Egyptian children and to study whether atopy is influenced by CD14 interaction with tobacco smoke exposure. CD14 –159 C/T and CD14 –550 C/T were genotyped in 500 asthmatic children, 150 allergic rhinitis children and 150 controls. We found that CD14 –159T allele, CD14 –550T allele and CD14 –159T/–550T haplotype were significantly associated with atopic asthma and allergic rhinitis groups. CD14 –159 TT and CD14 –550 TT genotypes associated with elevated IgE levels in children exposed to tobacco smoke. The TT genotype of CD14 –159 C/T and CD14 –550 C/T was associated with higher serum levels of sCD14. The present study indicated that CD14 gene polymorphisms may contribute to susceptibility to atopy in Egyptian children and influenced with tobacco smoke exposure.

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1. Introduction

Atopic diseases are characterized by an elevated and prolonged immunoglobulin E (IgE) antibody response after exposure to ubiquitous, nonpathogenic allergens [1]. Atopy is generally considered to be caused by the interaction of genetic and environmental factors [2]. The gene encoding CD14 is located in the chromosome region 5q31 [3], which have been found to be linked to atopy and asthma in several populations [4]. CD14 is a multifunctional receptor that is expressed on the surface of monocytes, macrophages and neutrophils, and as a soluble form in serum [5]. Soluble form (sCD14) is derived both from secretion of CD14 and from enzymatically cleaved glycosyl-phosphatidylinositol-anchored membrane CD14 (mCD14) [5]. CD14 is the principal receptor for lipopolysaccharides (LPS); whose engagement results in the activation of antigen presenting cells, including macrophages and dendritic cells [6]. The subsequent release of pro-inflammatory cytokines as IL-12 is regarded as an obligatory signal for the maturation of naive T cells into Th1 cells and the suppression of Th2 type cell-dependent IgE responses [7].

CD14 proteins are part of the receptor complex for endotoxin that is found in cigarette smoke particles [8]. Exposure to environmental tobacco smoke (ETS) results in the inhalation of endotoxin levels that are 120 times higher than exposure to smoke-free air [8]. Endotoxin is a potent inflammatory agent and may contribute to the high prevalence of respiratory disorders among smokers, and among nonsmokers exposed to ETS [9].

A genetic variant CD14 –159 C/T was found in the promoter region of the CD14 gene. The CD14 C –159T polymorphism was likely to have an age-related role in the development of atopy [10,11]. The functional significance of CD14 –159 C/T SNP in the susceptibility of asthma and atopy is unclear as the same allele has been associated with opposite effects in different studies [12]. It is possible that these variable associations with the SNP result from ethnic related differences. Inconsistent results may also be explained by the difference in environmental factors across studies, which may be important in modifying genetic associations [13]. Another CD14 polymorphism –550 C/T was associated with the serum level of sCD14 [14,15]; and its association with atopic phenotypes was examined but conflicting results have been observed [16,17]. Recent meta-analysis found the evidence for a relation of CD14 with childhood asthma is limited and future studies should include haplotype analysis and take environmental factors into account to further clarify the role of CD14 on childhood asthma [18]. So, in the present study, we investigate whether CD14 gene polymorphisms at positions –159 and –550 may contribute

Abbreviations: IgE, immunoglobulin E; sCD14, soluble form CD14; mCD14, membrane CD14; LPS, lipopolysaccharides; ETS, environmental tobacco smoke; RFLP, restriction fragment length polymorphism.

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to susceptibility to atopy in Egyptian children and also to study whether atopy is influenced by CD14 interaction with tobacco smoke exposure.

2. Patients and methods

The study was conducted by the medical biochemistry and pediatric departments at the faculty of medicine of Zagazig University – Egypt.

2.1. Patients

Two hundred fifty atopic children with asthma, 250 non-atopic children with asthma, and 150 children with allergic rhinitis were enrolled in this study. Following the American Thoracic Society guidelines, asthma was confirmed by a history of chest tightness and wheezing during the previous 12 months, a greater than 12% reversibility of forced expiratory volume (FEV1) spontaneously or after β_2 -agonist inhalation, and/or a methacholine provocation test results with a PC20 less than 16 mg/ml. Atopy was defined by a positive skin prick test (wheal diameter ≥ 3 mm) to at least one of 12 common aeroallergen. The diagnosis of allergic rhinitis and classification of the clinical severity was made according to Rhinitis scoring system [19]. None of the participants received antihistamine, systemic or topical corticosteroids in the 3 weeks prior to clinical evaluation, and they all underwent skin prick testing. Clinical phenotype was based on a questionnaire completed separately for each child by the parents. The questionnaire included demographics, medical history, skin-prick data, as well as ETS exposure was taken. ETS exposure was determined to be present if one or both parents or any other person in the household reported smoking during the first year of life.

2.2. Controls

The control group consisted of 150 children presented to the pediatric department of the Hospital of Zagazig University with other than respiratory or atopic complaints. The nonatopic control subjects had no history of asthma or other allergic diseases, negative skin prick tests, normal total IgE values (≤ 100 IU/ml), normal lung function tests, and no airway hyper-responsiveness (PC20 > 25 mg/ml). Patients and controls belonged to the same ethnic group: African Caucasian. The study was approved by the ethical committee of Faculty of Medicine, Zagazig University and written informed consent was obtained from all parents' participants. Characteristics of the participants are presented in Table 1.

3. Total IgE Measurements

Total serum IgE levels were measured by sandwich ELISA (Monobind Inc, Costa Mesa, California, USA), with an assay sensitivity for total IgE of 1.0 IU/ml.

4. sCD14 Measurements

Serum levels of sCD14 were measured by use of a commercially available ELISA kit (R&D Systems, Europe, Abdingdon, Oxon, United Kingdom), according to the manufacturer's instructions.

5. Genotyping

Genomic DNA was extracted from whole blood using a genomic DNA purification kit (Promega, Madison, Wisconsin, USA). Participants were genotyped for the SNP of CD14 gene –159 and –550 in genomic DNA.

Table 1
Clinical profiles of study subjects.

	Asthma group No = 500	Allergic rhinitis group No = 150	Control group No = 150
Age (years) [*]	8.6 $\pm$ 2.3	8.5 $\pm$ 3	10.2 $\pm$ 2.4
Male/female	268/232	75/65	77/73
BMI (kg/m ²)	18.4 $\pm$ 3.1	18.6 $\pm$ 2.9	17.9 $\pm$ 2.7
Mean age at the onset of disease (years) [†]	4.5	4.3	
FEV1 (%) [*]	87.5 $\pm$ 18	92.2 $\pm$ 16	97.5 $\pm$ 17
Log PC ₂₀ (mg/mL) [*]	0.31 $\pm$ 0.2	1.44 $\pm$ 0.5	1.36 $\pm$ 0.4
Log IgE (IU/mL) [*]	2.42 $\pm$ 0.6	2.25 $\pm$ 0.5	1.47 $\pm$ 0.6

^{*} $p < 0.05$ for differences among the three groups.

[†] $p < 0.05$ for difference between asthma and allergic rhinitis groups.

CD14 –159 C/T and –550 C/T polymorphisms were analyzed by means of PCR restriction fragment length polymorphism (RFLP) method according to [20,21]. The forward primer for CD14 –159 was 5'-GCCTCTGACAGTTTATGTAATC-3' and the reverse primer was 5'-GTGCCAACAGATGAGGTTTAC-3' while the forward primer for CD14-550 was 5'-GGAAGGGGGAATTTTCTTTAGGC-3' and the reverse primer was 5'-GGCAGTGTCTTGATGACTCA-3'. PCR was performed in a final volume of 25 μ L containing 3.5 μ L of H₂O, 5 μ L of genomic DNA, 1 μ L of each primer (1 μ M), and a 2 $\times$ Super Hot PCR Master Mix (12.5 μ L) (Bioron, Ludwigshafen am Rhein, Germany). The amplification was carried out using a PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts, USA). Cycling conditions were 94 $^{\circ}$ C for 3 min, 28 cycles of 94 $^{\circ}$ C for 30 s, 57 $^{\circ}$ C for 30 s, 72 $^{\circ}$ C for 30 s, and a final extension of 72 $^{\circ}$ C for 6 min for CD14 –159 C/T and 95 $^{\circ}$ C for 2 min, followed by 35 cycles of 95 $^{\circ}$ C for 30 s, 60 $^{\circ}$ C annealing for 30 s, 72 $^{\circ}$ C for 40 s and a final extension at 72 $^{\circ}$ C for 10 min for CD14 –550 C/T. PCR products were digested for 2 h at 37 $^{\circ}$ C (CD14 –159) or for 16 h at 37 $^{\circ}$ C (CD14 –550) in a 15 μ L reaction containing 5 U Aall restriction enzyme (New England Biolabs) for determination of CD14 –159 genotypes, or HaeIII (New England Biolabs) for determination of CD14 –550 genotypes, respectively. The final products were separated in 2% agarose gels. The digestion revealed fragments of 497 bp for the C allele of CD14 –159 and 144 and 353 bp for the T allele of CD-159 (Fig. 1). The C allele of the CD14 –550 appears as a single 236 bp band and the CD14 –550T allele as 258 bp fragments (Fig. 2).

6. Statistical analysis

The results for continuous variables are expressed as means $\pm$ SD. The means of the groups were compared by ANOVA-test. The statistical significances of differences in frequencies of variants between the groups were tested using the chi-square (χ^2) test. In addition, odds ratios (ORs) and 95% CIs were calculated as a measure of the association of the CD14 alleles with asthma and allergic rhinitis. Genotype frequencies in cases and controls were tested for Hardy-Weinberg's equilibrium, and any deviation between the observed and expected frequencies was tested for significance using the χ^2 test. Linear regression analysis was performed by stratifying patients based on ETS exposure. A difference was considered significant if p was < 0.05 . Data were analyzed using SPSS software version 11 (SPSS Inc, Chicago, Illinois, USA).

7. Results

The genotype frequencies of the CD14 –159 C/T and –550 C/T polymorphisms were in agreement with Hardy-Weinberg's equilibrium in all groups. There was no significant difference in genotype and allele frequencies between asthmatic children and

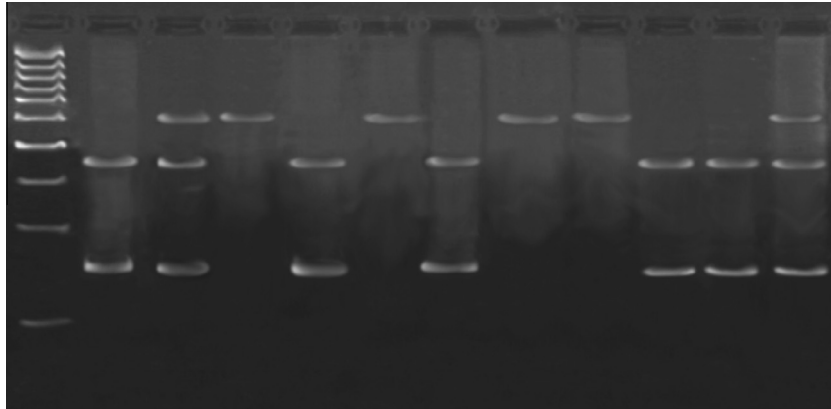


Fig. 1. Analysis of the CD14 –159 C/T polymorphism. Lane 1: DNA size marker (100 bp); lanes 2–12: patients with different CD14 –159 C/T genotypes; lanes 4, 6, 8 and 9: C allele (CC) homozygous; lanes 2, 5, 10 and 11: allele T (TT) homozygous; Lane 3, 7 and 12: (CT) heterozygous.

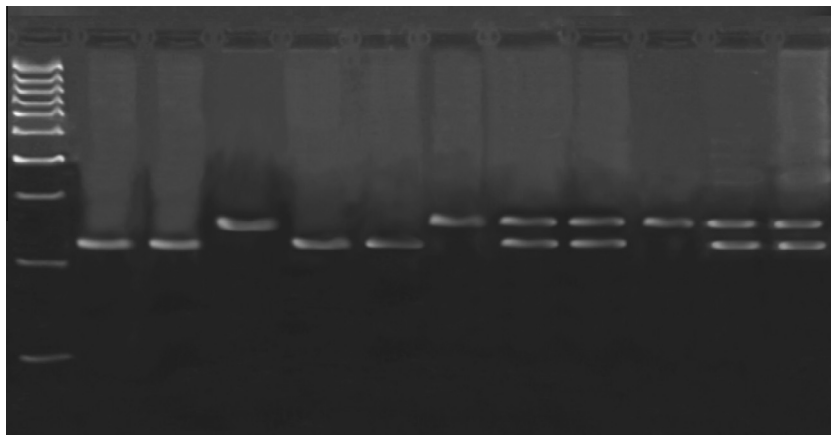


Fig. 2. Analysis of the CD14 –550 C/T polymorphism. Lane 1: DNA size marker (100 bp); lanes 2–12: patients with different CD14 –550 C/T genotypes; lanes 2, 3, 5 and 6: C allele (CC) homozygous; lanes 8, 9, 11 and 12: (CT) homozygous; lanes 4, 7 and 10: allele T (TT) heterozygous.

controls ($p = 0.09$, 0.1 for CD14 –159 and $p = 0.17$, 0.07 for CD14 –550 respectively). Asthmatic children were further stratified into two subgroups: those with atopy and those without atopy. There were significant differences between atopic asthma group and controls regarding the genotype and allele distributions of both CD14 –159 and –550 polymorphisms while there were no relevant differences between non atopic patients and controls. Prevalence of CD14 –159 TT genotypes and CD14 –159T alleles were significantly higher in asthmatic children with atopy as compared to controls ($p < 0.001$ for each) and the odds ratio and 95% CI for the T allele was 1.9 (1.4 – 2.6). Also, T genotypes and T alleles for CD14 –550 C/T was significantly associated with atopic asthma ($p = 0.006$, 0.002 respectively) and the odds ratio and 95% CI for the T allele of CD14 –550 C/T was 1.7 (1.2 – 2.3) (Table 2).

Concerning allergic rhinitis children, prevalence of CD14 –159 TT genotypes and CD14 –159T alleles were significantly higher as compared to controls ($p < 0.001$ for each) and the odds ratio and 95% CI for the T allele was 2.1 (1.5 – 2.9). For CD14 –550 C/T, TT genotypes and T alleles were significantly associated with the allergic rhinitis group ($p = 0.02$, 0.01 respectively) and the odds ratio and 95% CI for the T allele of CD14 –550 C/T was 1.6 (1.1 – 2.3) (Table 2).

Haplotypes were reconstructed in CD14 –159 C/T and –550 C/T polymorphisms, haplotype –159T/–550T (with all the variant alleles) was found to be associated with a higher risk of atopic asthmatic children [the odds ratio and 95% CI was 6.6 (3.3 – 13.4) and $p < 0.001$] and allergic rhinitis cases [the odds ratio and 95% CI was 5 (2.4 – 10.5) and $p < 0.001$]. On the other hand, frequency of

haplotype –159C/–550C (with all the wild alleles) was significantly higher in the control group as compared to atopic asthma [the odds ratio and 95% CI was 1.5 (1.2 – 2.1) and $p = 0.005$] and to allergic rhinitis [the odds ratio and 95% CI was 1.4 (1 – 1.9) and $p = 0.04$] (Table 3).

To test the possibility of an interaction between CD14 genotypes and ETS and atopic disease's susceptibility, atopic groups were stratified by ETS exposure; the TT genotypes frequencies of both polymorphisms were significantly differences in atopic asthmatic and allergic rhinitis children exposed to ETS compared to children not exposed to ETS (for CD14 –159 C/T; $p = 0.004$ in atopic asthma, $p < 0.001$ in allergic rhinitis group and for CD14 –550 C/T; $p = 0.008$ in atopic asthma, $p = 0.01$ in the allergic rhinitis group) (Table 4).

The effect of CD14 –159 C/T and –550 C/T polymorphisms on serum IgE level was investigated. There were no associations of both CD14 polymorphisms with IgE levels in all studied groups (Table 5). However, when we tested the influence of ETS in interaction with CD14 genotypes on total IgE levels, we found that the CD14 –159 TT genotype was associated with higher IgE levels in children exposed to ETS [geometric mean of IgE was 2.46 ± 0.2 (TT genotype) versus 2.34 ± 0.3 (CC + CT genotype), $p = 0.02$ in atopic asthmatics and 2.27 ± 0.2 versus 2.19 ± 0.1 , $p = 0.01$ in allergic rhinitis cases]. The same results were observed for CD14 –550 TT genotype [2.48 ± 0.2 (TT genotype) versus 2.33 ± 0.2 (CC + CT genotype), $p = 0.003$ in atopic asthmatics and 2.36 ± 0.1 versus 2.20 ± 0.2 , $p = 0.004$ in the allergic rhinitis group] (Table 6).

Table 2

Genotype and allele frequencies of CD14 –159 C/T and –550 C/T polymorphisms in groups studied.

	Control no (%)	Asthma group			Atopic asthma			Non-atopic asthma			Allergic rhinitis		
		No (%)	OR (95% CI)	<i>p</i> *	No (%)	OR(95% CI)	<i>p</i> *	No (%)	OR(95% CI)	<i>p</i> *	No (%)	OR (95% CI)	<i>p</i> *
<i>CD14 –159 genotype</i>													
CC	68 (45.3)	210 (42)		0.09	85 (34)		0.001	125 (50)		0.15	44 (29.3)		<0.001
CT	70 (46.7)	215 (43)			99 (39.6)			116 (46.4)			67 (44.7)		
TT	12 (8)	75 (15)			66 (26.4)			9 (3.6)			39 (26)		
<i>Allele</i>													
C	206 (68.7)	635 (63.5)	1	0.1	269 (53.8)	1	<0.001	366 (73.2)	1	0.17	155 (51.7)	1	<0.001
T	94 (31.3)	365 (36.5)	1.2 (0.9–1.7)		231 (46.2)	1.9 (1.4–2.6)		134 (26.8)	0.8 (0.6–1.1)		145 (48.3)	2.1 (1.5–2.9)	
<i>CD14 –550 genotype</i>													
CC	82 (54.7)	236 (47.2)		0.07	106 (42.4)		0.006	130 (52)		0.55	66 (44)		0.02
CT	60 (40)	219 (43.8)			108 (43.2)			111 (44.4)			63 (42)		
TT	8 (5.3)	45 (9)			36 (14.4)			9 (3.6)			21 (14)		
<i>Allele</i>													
C	224 (74.7)	691 (69.1)	1	0.07	320 (64)	1	0.002	371 (74.2)	1	0.88	195 (65)	1	0.01
T	76 (25.3)	309 (30.9)	1.3 (0.9–1.8)		180 (36)	1.7 (1.2–2.3)		129 (25.8)	1 (0.7–1.4)		105 (35)	1.6 (1.1–2.3)	

* *p* Calculated by chi square test. As compared to controls.**Table 3**

Haplotype frequencies of CD14 polymorphisms in atopic groups and controls.

Haplotype	Control No (%)	Atopic asthma			Allergic rhinitis		
		No (%)	OR (95% CI)	<i>p</i> [*]	No (%)	OR (95% CI)	<i>p</i> [*]
CD14 –159 –550							
CC	135 (45)	175 (35)	1.5 (1.2–2.1)	0.005	111 (37)	1.4 (1–1.9)	0.04
CT	108 (36)	160 (32)	0.9 (0.6–1.2)	0.25	102 (34)	0.9 (0.7–1.3)	0.61
TC	48 (16)	80 (16)	1 (0.7–1.5)	1	47 (15.7)	1 (0.7–1.5)	0.9
TT	9 (3)	85 (17)	6.6 (3.3–13.4)	<0.001	40 (13.3)	5 (2.4–10.5)	<0.001

* *p* As compared to control.**Table 4**

Genotype frequencies of CD14 polymorphisms according to environmental tobacco smoke (ETS) exposure in atopic groups.

Atopic asthma					Allergic rhinitis			
	ETS exposure No (%)	No ETS exposure No (%)	OR (95% CI)	<i>p</i>	ETS exposure No (%)	No ETS exposure No (%)	OR (95% CI)	<i>p</i>
CD14 –159								
CC + CT	83 (65.4)	101 (82.1)	2.4 (1.4–4.4)	0.004	37 (56.9)	74 (87.1)	5.1 (2.3–11.3)	<0.001
TT	44 (34.6)	22 (17.9)			28 (43.1)	11 (12.9)		
CD14 –550								
CC + CT	101 (79.5)	113 (91.9)	2.9 (1.3–6.3)	0.008	50 (76.9)	79 (92.9)	3.9 (1.4–10.9)	0.01
TT	26 (20.5)	10 (8.1)			15 (23.1)	6 (7.1)		

Table 5

Effect of CD14 gene polymorphisms on serum IgE levels in all studied groups.

		CC	CT	TT	<i>p</i> *
CD14 –159	Atopic asthma	2.37 ± 0.5	2.35 ± 0.6	2.42 ± 0.3	0.7
	Non-atopic asthma	1.5 ± 0.5	1.55 ± 0.7	1.6 ± 0.2	0.9
	Allergic rhinitis	2.23 ± 0.3	2.27 ± 0.3	2.24 ± 0.5	0.7
	Control group	1.44 ± 0.5	1.47 ± 0.3	1.47 ± 0.1	0.9
CD14 –550	Atopic asthma	2.39 ± 0.4	2.35 ± 0.2	2.44 ± 0.5	0.5
	Non-atopic asthma	1.48 ± 0.3	1.6 ± 0.5	1.46 ± 0.2	0.1
	Allergic rhinitis	2.24 ± 0.2	2.23 ± 0.4	2.28 ± 0.2	0.8
	Control group	1.45 ± 0.4	1.47 ± 0.4	1.48 ± 0.1	0.9

* Calculated by ANOVA test.

The CD14 –159 C/T and CD14 –550 C/T SNPs showed consistent and significant interaction with ETS exposure, with respect to serum IgE levels (*p* value for interaction in atopic asthma was

0.02 and 0.001 and in allergic rhinitis was 0.03 and 0.009 for CD14 –159 and –550, respectively) (Table 6).

It is important to show whether gene polymorphisms of CD14 have an effect on expression of its product; so, sCD14 levels have been measured by ELISA. The mean values of serum sCD14 were 2120.9 ± 450.3 ng/ml in controls, 3445.6 ± 558.8 ng/ml in patients with atopic asthma, 3160.9 ± 463.3 ng/ml in patients with non-atopic asthma, 3461.6 ± 500.2 ng/ml in allergic rhinitis children; and there was a significant increase of serum CD14 in atopic asthmatic, non-atopic asthma and allergic rhinitis children than in control (*p* < 0.001). Next, we investigated the association between the sCD14 levels and the allelic variants of CD14 polymorphisms; and we found that there was a significant increase in sCD14 levels in homozygous TT compared with homozygous CC or heterozygous CT of each polymorphism in all groups (Table 7). There were no associations of sCD14 levels with IgE levels in all studied groups (*p* > 0.05 for each group). Also, there was no effect of ETS exposure

Table 6

Influence of environmental tobacco smoke in interaction (ETS) with CD14 genotypes on total immunoglobulin E (IgE) levels in atopic groups.

	SNP	Genotype	ETS exposure	<i>p</i>	No ETS exposure	<i>p</i>	* <i>p</i> -Value interaction (ETS exposure versus no exposure)
Atopic asthma	CD14 –159	CC + CT	2.34 ± 0.3	0.02	2.38 ± 0.3	0.9	0.02
		TT	2.46 ± 0.2		2.37 ± 0.1		
	CD14 –550	CC + CT	2.33 ± 0.2	0.003	2.41 ± 0.4	0.8	0.001
		TT	2.48 ± 0.2		2.38 ± 0.4		
Allergic rhinitis	CD14 –159	CC + CT	2.19 ± 0.1	0.01	2.30 ± 0.3	0.4	0.03
		TT	2.27 ± 0.2		2.22 ± 0.3		
	CD14 –550	CC + CT	2.20 ± 0.2	0.004	2.27 ± 0.4	0.8	0.009
		TT	2.36 ± 0.1		2.22 ± 0.1		

* From linear regression analysis.

Table 7

Effect of CD14 gene polymorphisms on serum sCD14 levels in all studied groups.

		CC	CT	TT	<i>p</i> *
CD14 –159	Atopic asthma	3225.8 ± 512.2	3320.7 ± 530.2	3531.5 ± 380.2	<0.001
	Non-atopic asthma	3000.4 ± 312.3	3013.9 ± 401.5	3310.5 ± 210.9	0.04
	Allergic rhinitis	3289.2 ± 467.8	3279.5 ± 490.2	3541.9 ± 370.3	0.01
	Control group	2050.9 ± 340.5	2061.4 ± 350.4	2412.7 ± 300.2	0.003
CD14 –550	Atopic asthma	3356.7 ± 529.4	3342.8 ± 514.6	3603.4 ± 379.5	0.02
	Non-atopic asthma	3012.6 ± 320.5	3020.8 ± 435.4	3390.8 ± 256.8	0.01
	Allergic rhinitis	3200.7 ± 481.3	3210.4 ± 454.7	3567.8 ± 381.2	0.004
	Control group	2005.2 ± 350.6	2012.9 ± 361.1	2450.4 ± 350.2	0.003

* Calculated by ANOVA test.

on sCD14 levels when comparing between children exposed to ETS versus children not exposed to tobacco smoke (the mean values of sCD14 was 3460.5 ± 560.3 ng/mL versus 3430.4 ± 555.7 ng/mL, *p* = 0.67 and was 3452.3 ± 492.4 ng/mL versus 3465.5 ± 503.4 ng/mL, *p* = 0.87 for atopic asthma group and allergic rhinitis group respectively.

8. Discussion

We found that polymorphisms in CD14, which is a major player in the process leading to the detection of and development of biological responses against environmental microbial products, could be involved in a mechanism that resulted in susceptibility to atopy. This study demonstrated that minor alleles of CD14 –159 C/T and CD14 –550 C/T were significantly associated with atopic asthma and allergic rhinitis groups but not with non atopic asthma children. The CD14 haplotypes –159T/–550T was also associated with atopic asthma and allergic rhinitis susceptibility. The TT genotype of CD14 –159 C/T and CD14 –550 C/T was associated with a higher serum level of sCD14. Although there were no significant differences of IgE levels between CD14 –159 and CD14 –550 genotypes, the CD14 –159 TT and CD14 –550 TT genotypes associated with elevated IgE levels in children exposed to tobacco smoke. Many studies in different populations reported that C –159T did not play a role in the development of atopic asthma as in studies from Australia [22], Germany [23], Korea [24], China [25], and Slovenia [26]. Others showed that T allele was associated with a lower risk for either total or specific IgE, atopy or asthma as in studies in Turkish [27], Chinese [28], French [29], and Koreans [30]. Recently, Yazdani et al. [31] did not find an association between the distribution of C –159T and serum immunoglobulin E level. Zhang et al. [32] performed a meta-analysis on all case-control CD14 and asthma association studies and suggested that C –159T could be a protective factor for atopic asthma in Asian populations. However, many studies agreed with our results in which T allele showed a higher risk for atopy as those were done by Ober et al. [33] in Hutterites, Kedda et al. [23] in Australian, Litonjua et al. [34] and Jackola et al.

[35] among Caucasian in America. Recently, Micheal et al. reported that there is a significant association between C –159T polymorphism and allergic rhinitis in Pakistani adults [36]. Concerning CD14 –550 polymorphism, Smit et al. [16] found that CD14 –550 (–651 in his study) polymorphism was associated with atopy prevalence among young Danish exposed to farm environments. Others showed that CD14 –550 might not play a major role in the pathogenesis of food allergy in Japanese children [17]. Liang et al. [37] found no significant difference in the genotype frequencies of this SNP between non-atopy and atopy groups in Singaporean Chinese. Moreover, CD14 –550 genotypes did not predict changes in lung function in laboratory animal workers [38].

We often observed conflicting results in studies of the genetics of complex traits. Some showed associations between a polymorphism and a disease; others failed to replicate the results. Discrepancies may be due to differences in the populations studied, difficulty in defining the phenotypes, or lack of statistical power. Surprisingly, all the studies which agreed with our results shared one common feature, which is high environmental exposure as most of them occurred in rural areas. Other studies tried to find a link between polymorphism and environment as those assessing house dust endotoxin levels [39], having a pet in the house at birth [40], working with laboratory animals [41], contact with farm animals [42], and country living during childhood [43]. They found that, the –159T allele is associated with asthma or allergic disease among the highly “exposed” subjects, whereas the –159C allele is associated with asthma or allergic disease among “non-exposed” subjects. No associations with this polymorphism were seen among subjects with intermediate levels of exposure [13]. These findings suggested that there could be an antagonistic interaction between environmental contexts and CD14 –159 as determinants of allergic sensitization: the T allele could be either protective or a risk factor, depending on the degree of exposure to environmental microbial products, which we surmised could be present in different concentrations in urban and rural settings [44]. That's why, Braun-Farlander and other investigators of the Asthma and Endotoxin (ALEX) study [45] observed that, in children living in rural areas of German-speaking Western Europe, risk of allergic

sensitization was inversely associated with the concentration of endotoxin in house dust. And also made Martinez [46] hypothesized that higher CD14 expression in TT homozygote's increased sensitivity to the defensive effects of low level endotoxin exposure compared to carriers of other genotypes. However, at higher levels of endotoxin exposure induced CD14 expression could be increased in carriers of the C allele, showing a reversed protective effect. An interaction between CD14 –550 polymorphism and living in an Eastern vs. Western environment was associated with rhinitis in the Karelian children [47]. Rylander et al. [48] suggested that CD14 –550 polymorphism influence regulators of the inflammation induced by endotoxin in organic dusts.

Given the role of CD14 as a receptor for endotoxin/LPS, it was plausible to surmise that differential exposure to endotoxin could explain the heterogeneity of the results revealed by the different studies. This also explain our results concerning elevated IgE levels in the CD14 –159 TT and CD14 –550 TT genotypes among children exposed to tobacco smoke, which is considered another environmental risk factor. Our results are consistent with Choudhry et al. results [49], where in both studies; the association between the CD14 gene and IgE levels was only dependent on ETS exposure. However, in their study the increased IgE level was associated with the C allele not the T allele. There is reversed protective effect between the C and T allele according to the endotoxin exposure which may explain the difference between our and their results. The cause of increased IgE may be as Vercelli and coworkers showed that IL-4-dependent synthesis of IgE in peripheral-blood mononuclear cells was strongly up regulated by LPS, but down regulate in the presence of anti-CD14 [50]. In contrary of our results, no gene–environment interactions of ETS exposure with CD14 –159 and –550 SNPs were found with respect to sensitisation to egg or milk allergens at ages 1 and 2 years, and indoor allergens at ages 4 and 8 years [40].

We found that the TT genotype of CD14 –159 C/T and CD14 –550 C/T was associated with a higher serum level of sCD14. Previous studies reported that TT homozygotes of CD14 –159 had significantly higher sCD 14 levels than did carriers of the CC genotypes in the populations of the Tucson Children's Respiratory Study (CRS) [51], German [52], Chinese [53] and Australian [54]. However, in two relatively small population studies, no association was found between the SNP and sCD14 [55,56], which may be on account of the lack of sufficient statistical power or differences in ethnicity.

The present study's finding of a significant increase in CD14 levels in homozygous TT compared with homozygous CC of CD14 –550 was different from other studies that showed TT homozygous seemed to have lower sCD14 levels when compared to CC [14,15]. An interesting study found that CD14 –550 were associated with sCD14 levels at birth, 2 years and 10 years [57]. These discrepancies might be explained by several factors related to the subjects, such as their age or ethnic composition, or to selection criteria.

In conclusion, CD14 gene polymorphisms may contribute to susceptibility to atopy in Egyptian children. Furthermore, atopy was importantly influenced by CD14 in interaction with tobacco smoke exposure.

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