

Association Between *CD14* Polymorphism (−1145G/A) and Childhood Bronchial Asthma

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Abstract Polymorphisms in the promoter region of *CD14* gene have been associated with asthma and atopy although the findings between cohorts have not been uniform. We aimed at investigating the association between *CD14* gene (−1145G/A) polymorphism and bronchial asthma in Egyptian children. Genotyping of *CD14* gene (−1145G/A) polymorphism was done by real-time PCR in 192 asthmatic children (atopic, $n = 100$ and non-atopic, $n = 92$) and 181 age- and gender-matched healthy children. Serum levels of total IgE were measured by ELISA. Skin prick test was performed on all patients. We found that the frequency of AA genotype was significantly higher in asthmatic children compared to healthy controls. Asthmatic children carrying GG genotype had a significantly lower prevalence of atopic asthma. Meanwhile, the “A” allele was significantly higher in atopic asthmatic children compared to healthy and non-atopic children. Moreover, atopic children carrying the “G” allele showed better asthma control. In conclusion, our findings represent an evidence for the role of *CD14* gene (−1145G/A) polymorphism in childhood asthma and asthma control.

Keywords Asthma · *CD14* · IgE · Polymorphism

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Introduction

Asthma results from interactions between environmental and genetic factors and is considered by far the commonest of all chronic diseases of childhood (Pinto Pereira et al. 2010). In developed countries, asthma was estimated to affect between 11 and 20% of all school age children (Clark et al. 1995), while the prevalence of asthma among Egyptian children aged 3–15 years was estimated to be 8.2% (Salama et al. 2010). Moreover, in Egypt, up to one in four children with asthma is unable to attend school regularly because of poor control of asthma (Bassili et al. 2000).

Cluster of differentiation 14 (*CD14*) gene is located on chromosome 5q31.3, a region reported to be associated with asthma (Bouzigon et al. 2010) and loci regulating total serum immunoglobulin E (IgE) (Baldini et al. 1999). It encodes for a protein which functions as a component of the innate immune system (Ulevitch and Tobias 1995). The CD14 receptor is of high specificity for lipopolysaccharides endotoxins and it forms a complex with toll-like receptor which plays an important role in allergic reactions in the respiratory tract. The interaction between CD14 and toll-like receptors induces the release of pro-inflammatory cytokines as interleukin-1 and tumor necrosis factor alpha responsible for the characteristics of bronchial asthma (Yamashita and Nakayama 2008).

There is a controversy regarding the association between genetic polymorphisms in the *CD14* gene and asthma. While multiple studies found that polymorphisms in the promoter region of *CD14* have been associated with asthma and hypersensitivity disease in Americans, Czech, Latin American, Japanese, and Korean (Baldini et al. 1999; Choudhry et al. 2005; Hong et al. 2007), another study found no evidence of a role for any single nucleotide polymorphism (SNP) in the *CD14* gene and the risk of childhood asthma among Caucasian children (Klaassen et al. 2013). Furthermore, IgE synthesis is controlled by the cytokines produced by T-helper (Th)1 and Th2 lymphocytes, and the study done by Baldini and his co-workers demonstrated that altered CD14 expression affects the proportion of Th1 to Th2 cells, thereby influencing IgE responses and associated inflammatory phenotypes in asthma (Baldini et al. 1999).

Therefore, we selected *CD14* gene (−1145G/A) variant, which is known to have a specified extent for asthma and/or atopic diseases (Wuthrich et al. 1992; Bucková et al. 2003), to evaluate its association with asthma in Egyptian children. Moreover, its relation to total IgE and the possibility of its role in atopic childhood asthma were studied.

Materials and Methods

This study included 192 asthmatic children (atopic, $n = 100$ and non-atopic, $n = 92$) recruited from the Pediatric Chest Clinic, Children's Hospital, Ain Shams University. They were diagnosed according to the clinical manifestations (GINA 2008) and confirmed by spirometry according to the standards of the European Respiratory Society and the American Thoracic Society (Miller et al. 2005). Age-

and gender-matched non-atopic, non-asthmatic healthy children ($n = 181$) were chosen as controls. They were selected from the geographic area surrounding the place of study. There was no significant difference in school grade between children participating in the study.

Skin prick tests (SPTs) were performed on all patients. Atopy was defined as at least one positive SPT result for common inhalant allergens (with positive histamine prick result). It was carried out using stainless-steel lancets. The panel used was the Taiwan Asian panel (as it was found to be the most matching panel to Egyptians) that includes House dust Mite *Farinae*, Mite *pteron*, Cockroach Mix, Feather Mix, Dogs' and Cats' dander, *Candida*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Alternaria*, Grass Mix, *Bremuda* Grass, Wheat, Cod fish, Pork, Beef, Egg, Whole, Egg Yolk, Milk, Yeast Brewer, Soybean, Peanut, Vegetable Mix, Rag wd Mix II, Pine Mix, Cotton wd, Mulberry Mi, Pigweed Mix, Corn, Crab, Shellfish Mix, Shrimp, and Eucalyptus (Hamid et al. 2009; Morcos et al. 2011). Histamine solution (10 mg/ml) and glycerinated saline were used as positive and negative controls. SPTs were considered positive if there was a wheal of 3 mm in diameter or larger.

This study has complied with the principles laid down in the Declaration of Helsinki, adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, and recently amended at the 59th World Medical Assembly, Seoul, Korea, October 2008. The entire protocol was approved by our institutional ethical committee. All parents or care givers provided signed informed consent for participation in the study as required.

Blood Sample Collection and Processing

Five milliliter of venous blood sample was withdrawn from each participant under complete aseptic conditions and divided into two portions as follows: 2.0 ml of blood was placed in an EDTA-containing tube for genotyping of *CD14* gene ($-1145G/A$) (*rs 2569190*) polymorphism and the remaining 3.0 ml of blood was used for separation of serum. The separated serum samples were kept frozen at -80°C until used in the quantitative determination of total IgE according to the manufacturer's instructions of the human total IgE ELISA kit (General Biologicals Corporation, Taiwan).

DNA was extracted using QIAamp DNA blood mini kit (QIAGEN, Valencia, CA). The DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm. Following DNA extraction, a 200 μl sample yielded 6 μg of DNA (30 $\text{ng}/\mu\text{l}$) with an A260/A280 ratio of 1.7–1.9. Extracted DNA was stored at -80°C until used.

Genotyping of *CD14* Gene ($-1145G/A$) Polymorphism

The genotypes were determined using TaqMan *CD14* (*rs 2569190*) kit (ABI/Life Technologies, USA). Briefly, PCR reaction mix/sample contained 10 μl universal TaqMan PCR Master Mix (2 $\times$), 0.5 μl *CD14* forward primer, 0.5 μl *CD14* reverse primer, 0.5 μl *CD14* probe A allele, 0.5 μl *CD14* probe G allele, 0.5 μl DNA extract

containing 20 ng in a 20 μ l final volume of DNase/RNase-free water. Following polymerase activation at 95°C for 10 min, amplification was performed by 40 cycles of denaturation at 95°C for 15 s, annealing/extension at 60°C for 60 s. The normalized (Rn) FAM fluorescence (SNP G allele) and the normalized (Rn) VIC fluorescence (SNP A allele) signal were monitored continuously during the PCR cycles. Amplification and allelic discrimination plots of the A and G alleles were constructed based on fluorescent FAM and VIC release of the TaqMan probes for the G and A alleles, respectively. Based on amplification and allelic discrimination plots, automatic genotyping decisions were made by the Applied Biosystem software (Fig. 1).

Statistical Analysis

The analysis was done using the Statistical Package for the Social Sciences (SPSS software version 19, SPSS Inc., Chicago, IL). Single nucleotide polymorphism was assessed for genotypic association analysis among asthmatic and healthy children. The genotype data of the tested SNP was then used to estimate Hardy–Weinberg equilibrium by comparing genotype frequencies within groups by a χ^2 -test. Fisher's exact test was applied to analyze the comparison of the frequencies of discrete

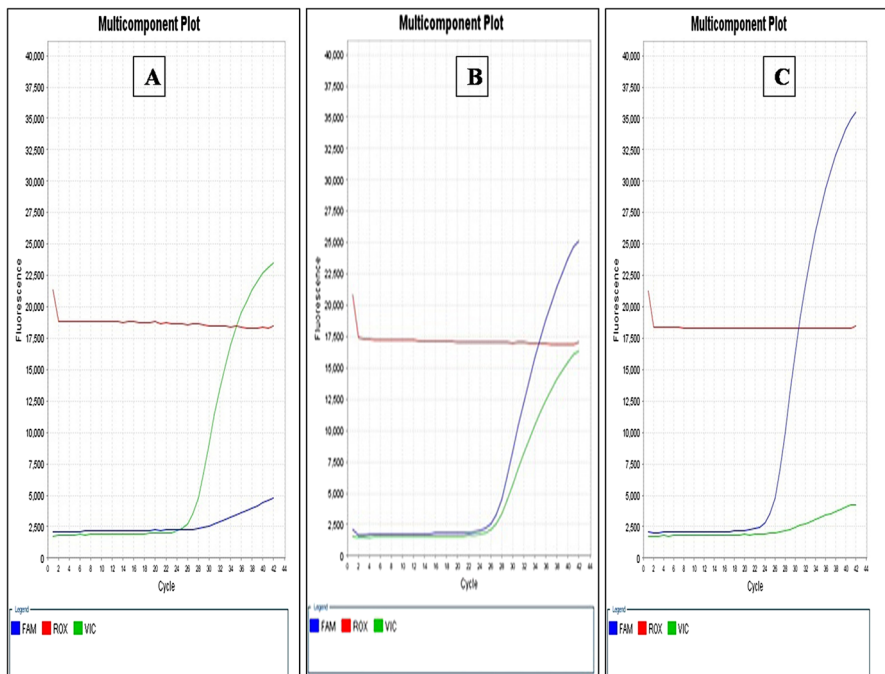


Fig. 1 **a** Representative amplification curve of CD14 ($-1145G/A$, $rs\ 2569190$) genotype AA (Homozygous VIC). **b** Representative amplification curve of CD14 ($-1145G/A$, $rs\ 2569190$) genotype AG (Heterozygous FAM, VIC). **c** Representative amplification curve of CD14 ($-1145G/A$, $rs\ 2569190$) genotype GG (Homozygous FAM)

variables between asthmatic and healthy children. The odds ratios (ORs) with 95% confidence intervals (95% CI) were also calculated to estimate risk of asthma associated with the *CD14* gene (*-1145G/A*) polymorphic genotypes. The OR is the ratio of the odds of exposure among the cases (numbers exposed divided by numbers not exposed) to the odds in favor of exposure among the controls. The ORs were calculated using the SPSS software. Comparison between two groups using Student's *t* test was done for continuous variable. Using the Non-Parametric Kruskal–Wallis (χ^2) test, the mean ranks and medians of serum total IgE were estimated. The sample size was calculated using a single population proportion formula described by Kirby et al. 2002. Statistical significance was set at a value of $p < 0.05$. * $p < 0.05$ is significant, ** $p < 0.01$ is highly significant.

Results

The clinical characteristics of asthmatic and healthy children are shown in Table 1. Genotype frequencies in asthmatic children ($p = 0.990$) and healthy children ($p = 0.398$) were consistent with Hardy–Weinberg equilibrium. The mean ranks (75.50, 39.68, and 21.32 for atopic, non-atopic, and healthy children, respectively) and median levels of total IgE (344.50, 99.50, and 64.50 for atopic, non-atopic, and healthy children, respectively) were significantly higher in atopic asthmatic children ($p < 0.001$) compared to healthy and non-atopic asthmatic children.

Table 1 Clinical characteristics of asthmatic and healthy children

Characteristics	Asthmatic children (<i>n</i> = 192)	Healthy children (<i>n</i> = 181)	<i>p</i> value
Age (mean $\pm$ SD) years	7.86 $\pm$ 3.20	8.35 $\pm$ 2.19	0.323
Total frequency of males (number, percentage)	132 (68.7%)	126 (69.6%)	1.000
Total frequency of females (number, percentage)	60 (31.3%)	55 (30.4%)	1.000
BMI (mean $\pm$ SD)	19.9 $\pm$ 1.61	20.1 $\pm$ 1.20	0.376
FEV ₁ (% of predicted $\pm$ SD)	92.29 $\pm$ 17.15	104.1 $\pm$ 10.44	<0.001**
FVC (% of predicted $\pm$ SD)	97.78 $\pm$ 17.14	105.59 $\pm$ 14.28	0.048*
FEV ₁ /FVC (% of predicted $\pm$ SD)	96.18 $\pm$ 14.66	103.61 $\pm$ 18.51	0.043*
MMEF (25–75% of predicted $\pm$ SD)	71.51 $\pm$ 21.12	88.20 $\pm$ 11.70	<0.001**
Passive smoking (number, percentage)			
Positive	96 (50%)	55 (30.4%)	0.120
Negative	96 (50%)	126 (69.6%)	
Family history of asthma (number, percentage)			
Positive	96 (50%)	0 (0%)	<0.001**
Negative	96 (50%)	181 (100%)	

BMI body mass index, *FEV₁* forced expiratory volume in the first second, *FVC* forced vital capacity, *MMEF* maximum mid-expiratory flow

* $p < 0.05$ is significant, ** $p < 0.01$ is highly significant

The frequency of AA genotype was significantly higher in asthmatic children ($n = 88$, 45.8%, $\chi^2 = 5.394$, OR 2.855, 95% CI 1.159–7.036, $p = 0.018$) and atopic asthmatic children ($n = 78$, 78.0%, $\chi^2 = 16.246$, OR 6.052, 95% CI 2.374–15.424, $p < 0.001$) compared to healthy children ($n = 53$, 29.3%). Also, its frequency was significantly higher in atopic asthmatic children compared to non-atopic asthmatic children ($n = 10$, 10.9%, $\chi^2 = 24.451$, OR 11.523, 95% CI 3.899–34.053, $p < 0.001$). Meanwhile, the frequency of AG genotype was significantly higher in non-atopic asthmatic children ($n = 70$, 76.1%, $\chi^2 = 5.443$, OR 3.341, 95% CI 1.170–9.538, $p = 0.026$) compared to atopic asthmatic children ($n = 14$, 14%). Regarding GG genotype, higher incidence was detected in healthy controls (18.2%) and non-atopic children (13%) compared to atopic children (8%), Table 2.

By comparing the allelic frequencies of *CD14* gene (–1145G/A) among the studied groups, we found that “A” allele was significantly higher in asthmatic children ($n = 260$, 67.7%, $\chi^2 = 3.351$, OR 1.708, 95% CI 0.961–3.034, $p = 0.046$) and atopic asthmatic children ($n = 170$, 85.0%, $\chi^2 = 20.960$, OR 4.554, 95% CI 2.319–8.941, $p < 0.001$) compared to healthy children ($n = 201$, 55.5%). Moreover, the frequency of “A” allele was significantly higher in atopic asthmatic children compared to non-atopic children ($n = 90$, 48.9%, $\chi^2 = 29.308$, OR 5.898, 95% CI 3.004–11.579, $p < 0.001$), Table 3.

Regarding total IgE, we noticed that it differed significantly among the three *CD14* gene (–1145G/A) polymorphic genotypes in children with atopic asthma and the highest median levels were detected in AA (395 IU/ μ l) and AG (340 IU/ μ l) genotypes compared to GG genotype (210 IU/ μ l, $p = 0.017$ for AA vs GG and $p = 0.025$ for AG vs GG), Table 4.

When we studied the relation between genotypes and allelic frequencies of *CD14* gene (–1145G/A) and asthma control in atopic asthmatic children, we found that atopic children carrying the “G” allele showed better asthma control, Table 5.

Discussion

This study reports a significant association between the *CD14* (–1145G/A) gene polymorphism and the risk of atopic childhood asthma in Egyptian children. It was specifically noticed that children carrying GG genotype had a significantly lower prevalence of atopic asthma. Meanwhile, the “A” allele was significantly higher in atopic asthmatic children compared to healthy and non-atopic children. These findings represent an evidence for the role of *CD14* in childhood asthma.

The results of previous studies investigating relationships between *CD14* (–1145G/A) polymorphism and asthma or allergy demonstrated considerable variation in the direction of associations and the significant genotypes involved. Similar to our study, Micheal et al. (2011) observed that *CD14* (–1145G/A) polymorphism was involved in various atopic conditions specifically atopic asthma ($p = 0.02$). However, they found that G allele was significantly higher in patients with atopic asthma (60%) than in controls (47%). Contrary to our results, Wang et al. (2014) did not find any relation between genotypes of *CD14* (–1145G/A)

Table 2 Genotype distribution of CD14 (−1145G/A) polymorphism between study groups

Genotype	Asthmatic children (n = 192)	Children with atopic asthma (n = 100)	Children with non-atopic asthma (n = 92)	Healthy children (n = 181)	Asthmatic children versus healthy children		Children with atopic asthma versus healthy children	
					OR (95% CI)	χ^2	p value	p value
AA (n, %)	88 (45.8%)	78 (78%)	10 (10.9%)	53 (29.3%)	2.855 (1.159–7.036)	5.394	0.018*	16.246 <0.001**
AG (n, %)	84 (43.8%)	14 (14%)	70 (76.1%)	95 (52.5%)	1.494 (0.626–3.568)	0.824	0.246	1.011 0.229
GG (n, %)	20 (10.4%)	8 (8%)	12 (13%)	33 (18.2%)	1 (ref.) ^a			
Genotype	Asthmatic children (n = 192)	Children with atopic asthma (n = 100, %)	Children with non-atopic asthma (n = 92, %)	Healthy children (n = 181)	Children with non-atopic asthma versus healthy children		Children with atopic asthma versus children with non-atopic asthma	
					OR (95% CI)	χ^2	p value	p value
AA (n, %)	88 (45.8%)	78 (78%)	10 (10.9%)	53 (29.3%)	0.477 (0.173–1.314)	2.079	0.118	24.451 <0.001**
AG (n, %)	84 (43.8%)	14 (14%)	70 (76.1%)	95 (52.5%)	1.985 (0.897–4.396)	2.919	0.066	5.443 0.026*
GG (n, %)	20 (10.4%)	8 (8%)	12 (13%)	33 (18.2%)	1 (ref.) ^a			

* $p < 0.05$ is significant, ** $p < 0.01$ is highly significant

^a GG genotype as reference (OR 1.0)

Table 3 Allelic association analysis of CD14 (−1145G/A) polymorphism between study groups

Allele	Asthmatic children (<i>n</i> = 192)	Children with atopic asthma (<i>n</i> = 100, %)	Children with non-atopic asthma (<i>n</i> = 92, %)	Healthy children (<i>n</i> = 181)	Asthmatic children versus healthy children		Children with atopic asthma versus healthy children	
					OR (95% CI)	χ^2	<i>p</i> value	χ^2
A (<i>n</i> , %)	260 (67.7%)	170 (85%)	90 (48.9%)	201 (55.5%)	1.708 (0.961–3.034)	3.351	0.046*	20.960
G (<i>n</i> , %)	124 (32.3%)	30 (15%)	94 (51.1%)	161 (44.5%)	1 (ref.) ^a			1 (ref.) ^a
Allele	Asthmatic children (<i>n</i> = 192)	Children with atopic asthma (<i>n</i> = 100, %)	Children with non-atopic asthma (<i>n</i> = 92, %)	Healthy children (<i>n</i> = 181)	Children with non-atopic asthma versus healthy children		Children with atopic asthma versus children with non-atopic asthma	
					OR (95% CI)	χ^2	<i>p</i> value	χ^2
A (<i>n</i> , %)	260 (67.7%)	170 (85%)	90 (48.9%)	201 (55.5%)	0.772 (0.443–1.344)	0.837	0.220	29.308
G (<i>n</i> , %)	124 (32.3%)	30 (15%)	94 (51.1%)	161 (44.5%)	1 (ref.) ^a			1 (ref.) ^a

* *p* < 0.05 is significant, ** *p* < 0.01 is highly significant

^a G allele as reference (OR 1.0)

Table 4 Relation between genotypes of CD14 (–1145G/A) polymorphism and median levels of total IgE (IU/μl) in different groups of the study

Groups	Total IgE (IU/μl)	Genotypes			<i>p</i> value
		AA	AG	GG	
Children with atopic asthma	Median (range)	395 (201–400)	340 (245–395)	210 (201–220)	p1 = 0.901
	Mean rank	14.09	13.60	3.50	p2 = 0.017*
					p3 = 0.025*
Children with non-atopic asthma	Median (range)	105 (90–112)	100 (59–115)	79.5 (63–102)	p1 = 0.445
	Mean rank	16.67	13.09	10.13	p2 = 0.077
					p3 = 0.289
Healthy children	Median (range)	67 (50–93)	65 (47–85)	63.5 (43–95)	p1 = 0.426
	Mean rank	14.56	12.13	11.60	p2 = 0.462
					p3 = 0.804

p1 = AA versus AG, p2 = AA versus GG and p3 = AG versus GG

* *p* < 0.05 is significant

Table 5 Relation between genotypes of CD14 (–1145G/A) and asthma control in atopic asthmatic children

Parameters	Atopic asthmatic children subgroups (n, %)		<i>p</i> value
	Controlled (n = 12, 12.0%)	Uncontrolled (n = 88, 88.0%)	
Spirometry data			
FEV ₁ (% of predicted ± SD)	95.94 ± 16.93	86.26 ± 16.29	0.043*
FVC (% of predicted ± SD)	103.14 ± 13.25	88.20 ± 12.43	0.034*
FEV ₁ /FVC (% of predicted ± SD)	100.08 ± 14.51	89.43 ± 13.35	0.021*
MMEF(25–75% of predicted ± SD)	76.52 ± 18.89	58.61 ± 21.47	0.006**
Genotype (n, %)			
AA	2 (2.6%)	76 (97.4%)	<0.001**
AG	2 (14.2%)	12 (85.8%)	<0.001**
GG	8 (100.0%)	0 (0.0%)	<0.001**
Allele (n, %)			
A allele	6 (3.5%)	164 (94.5%)	<0.001**
G allele	18 (60.0%)	12 (40.0%)	<0.001**

FEV₁ forced expiratory volume in the first second, FVC forced vital capacity, MMEF maximum mid-expiratory flow

* *p* < 0.05 is significant, ** *p* < 0.01 is highly significant

polymorphism and asthma. The discrepancy between our findings and those of previously published studies can be attributed to the variation of genotype frequencies of genes according to population and ethnicity.

In this study, it was not surprising that median level of total IgE was significantly higher in children with atopic asthma compared to children with non-atopic asthma and healthy controls. This result confirmed the usefulness of total IgE as indicator of atopic diseases (Barnes 2009; Gergen et al. 2009). Importantly, our study found that children with either the AA or AG variants of *CD14* (−1145G/A) polymorphism were more likely to have higher IgE levels compared to those of the GG variant. The data we found do suggest that *CD14* (−1145G/A) GG variant could play a protective role against atopic childhood asthma.

These findings were in agreement with another study which found that carriers of −1145A/haplotype had the highest levels of IgE and the lowest levels of sCD14, and conversely, carriers of the −1145G/haplotype had the highest levels of sCD14 and the lowest IgE values (Vercelli et al. 2001).

Our findings add to current understanding of the potential importance of the *CD14* SNPs for asthma control. Previous studies regarding *CD14* (−1145G/A) polymorphism to date do not evaluate the association between *CD14* (−1145G/A) and lung capacity or asthma control. Our findings of polymorphisms associated with lung capacity or asthma control are novel. The higher response to therapy in children with the G allele suggests that children with the G allele show better prognosis than those having the A allele.

In conclusion, the elucidation of the precise role of *CD14* (−1145G/A) polymorphism in the pathogenesis of atopic childhood asthma has the potential to have profound effects on the ability to prevent and treat this disorder. In this study, we have reported a novel association between the *CD14* (−1145G/A) gene polymorphism and the risk of atopic childhood asthma. Further studies on a large cohort of patients will be necessary to confirm these findings. Along with other known asthma risk genes, the addition of *CD14* (−1145G/A) gene involvement in the understanding of asthma/atopy pathogenesis will shed light for better control and treatment.

Compliance with Ethical Standards

Conflict of Interest None.

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