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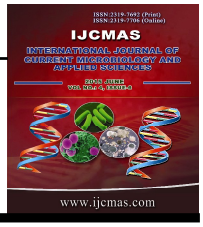

Original Research Article

Genetic Basis of Childhood Asthma in Saudi Arabia

Adnan A. Alsulaimani[1], Nabil S. Awad[2], and Adel E. El-Tarras[3,4*]

[1]College of Medicine, Taif University, Taif, Kingdom of Saudi Arabia
[2]Department of Genetics, Faculty of Agriculture and Natural Resources, Aswan University, Egypt
[3]Biotechnology and Genetic Engineering Unit, Scientific Research Deanship, Taif University, Saudi Arabia
[4]Genetics Dep., Faculty of Agriculture, Cairo University, Cairo, Egypt

**Corresponding author*

Keywords

Asthma, Childhood Asthma, CD14 gene Saudi Arabia

A B S T R A C T

Asthma is one of the most common chronic illnesses in Saudi Arabia and showed regional diversity. Suggestive susceptibility genes have been identified in European and American populations but not yet in Middle East including Saudi Arabia. CD14 (cluster of differentiation 14) gene is one of many genes that appear to contribute to the expression of allergic phenotype. The present study was carried out to determine the prevalence of some CD14 promoter polymorphisms in childhood asthmatic patients in Saudi Arabia. Polymerase Chain Reaction and Restriction Fragment Length Polymorphism technique was adopted with 42 childhood asthmatic patients. C(-159)T, A(-1,145)G and G(-1,395)T polymorphisms of CD14 gene were genotyped. The prevalence of studied polymorphisms was 50%, 45.2% and 42.8%, respectively. In conclusion, high prevalence of studied polymorphisms C(-159)T, A(-1,145)G and G(-1,395)T was observed in tested Saudi asthmatic childhood patients.

Introduction

Asthma is the most common chronic disease in childhoods. Asthma is affecting approximately 300 million individuals worldwide. It is a chronic inflammatory disorder of the airway with associated bronchoconstriction leading to airflow obstruction (Ho, 2010). Asthma is main reason of chronic illness in children and, for unknown reasons, is progressively increasing (Galassi *et al.*, 2006; Bateman and Jithoo 2007; Pearce *et al.*, 2007). An increase in both the prevalence and morbidity of asthma, especially in children younger than 6 years was recorded. Newacheck and Halfon (2000) and Al-Dawood (2002) reported that bronchial asthma (BA) during childhood is considered to have been the single most prevalent cause of childhood disability. In Saudi Arabia, asthma is a common chronic illness (Al-Moamary *et al.*, 2009). Saudi Arabia is considered one of the countries that have

very high prevalence of BA in children (Al Frayh *et al.,* 2001) and it also, showed regional diversity. The variations in the rate of prevalence of asthma are due to environmental and geographical influences (Al Frayh and Hasnain, 2007). Abudahish and Bella (2006) pointed that there was a significant increase in the prevalence of bronchial asthma in Saudi Arabia from 8% in 1986 to 23% in 1995. The changes in contemporary life may well have contributed to the increased prevalence of asthma, due to exposure to environmental factors such as tobacco smoke and indoor animals in Saudi houses. Also, poor knowledge, fear of use of new drugs, lack of awareness of the importance of control of the diseases are common among primary care physicians caring for asthma patients in the Saudi Arabia (Al-Moamary *et al.,* 2009; Speight *et al.,* 1983). Therefore, many asthma patients continue to be under diagnosed, under treated and at risk of acute exacerbations resulting in missing work or school and an increase in use of expensive acute healthcare services and reduce quality of life (Al-Moamary *et al.,* 2009; Al-Jahdali *et al.,* 2008).

In a recent asthma control survey cleared that only 5% were controlled, 31% were partial controlled and 64% were uncontrolled (Al-Moamary *et al.,* 2009; Dizier *et al.,* 2000). Prevalence of Questionnaire Diagnosed Asthma among children in school at Al-Khobar city, Saudi Arabia was more than that which was described earlier. However, this rate was less than those reported from other parts of Saudi Arabia, but higher than the ones reported from Arab developing countries and European countries. Xu *et al.* (2001) reported that there is evidence that genetic liability for asthma, airways responsiveness and allergic traits are regulated through distinct loci. The susceptibility genes have been identified in European and American populations but not yet in Middle East countries including Saudi Arabia.

Many researchers pointed out that multiple genome-wide linkage studies for asthma and allergy have been performed, and have identified more than 20 distinct chromosomal regions with linkage to asthma or related traits (Hakonarson *et al.,* 1997; Laitine *et al.,* 2001; Haagerup *et al.,* 2002; Weiss and Raby, 2004). Several of these linkages have been reported in more than one study; of which the most frequently reproduced include regions 6p, 5q, 12q and 13q. Furthermore, convincing evidence of linkage on chromosomes 14q and 7p has been observed in founder populations from Iceland and Finland, respectively (Hakonarson *et al.,* 1997; Al-Dawood 2001). The majority of these regions are large, spanning 10–30Mb and harboring hundreds of genes. Recently, mapping of these broad regions is difficult, due to the lack of availability of high-resolution linkage disequilibrium maps. Two novel asthma genes were identified by positional cloning. Denham *et al.* (2008) demonstrated that one of these genes (PHF11) appears to primarily influence total IgE level, and impact asthma severity through this intermediate phenotype. The second, DPP10, is more strongly associated with asthma, although variation at adjacent loci may independently affect atopy-related phenotypes. Recently, it is well accepted that asthma is a complex disease and both genetic and environmental factors contribute to its inception and evolution (Shapiro and Owen 2002; Li *et al.,* 2010; Zhang *et al.,* 2010). Possible hypotheses may be that different genetic backgrounds and environment exposures in different ethnic population may affect the pathogenesis of asthma. Van Eerdewegh *et al.* (2002) suggested that asthma susceptible genes in different population may not be the

same. Therefore, it is important to carry out genetic studies on different ethnic populations and in different countries.

The gene CD14 is one of many genes that appear to contribute to the expression of the allergic phenotype. The gene is also, responsible for recognition of bacterial lipopolysaccharides (LPS) in innate immunity. This gene is localized on chromosome 5q31.1, and it's a region linked to both asthma and total serum IgE concentration (Marsh *et al.* 1994; Meyers *et al.* 1994). Yazdani *et al.* (2012) added that CD14 gene is located on chromosome 5q31-32, which is considered a critical region for several allergic and atopic diseases, including asthma. Also, Vercelli *et al.* (2001) stated that innate immunity genes, which operate at the interface between the immune system and pathogens such as mite allergens, are believed to play a critical role in the development of allergic asthma. The gene D14, a membrane glycoprotein (mCD14) expressed on the surface of monocytes, macrophages, granulocytes, and B lymphocytes, is an important molecule of the innate immune system. This gene is functioning as a carrier and receptor for microbial ligands. Gene is also present as soluble CD14 in serum (Buckova *et al.*, 2003). Holgate (1999) mentioned that, upon binding, CD14 signals enhance production of interleukin (IL) 12, which is in turn required for maturation of naïve T cells into type 1 helper T cells (TH1), down-regulation of TH2 cells, and subsequent decreased immunoglobulin (Ig) E production. Polymorphisms in the CD14 promoter region have been associated with atopic diseases and IgE levels. Five major genetic variants have been identified in the promoter of the CD14 gene (C–159T, A–1619G, G–1359T, A–1145C, and A–809C) and are associated with atopic phenotypes in different ethnic groups (Baldini *et al.*, 1999). It has been showed that carriers of the _1,359T/_1,145A/_159C haplotype had highest levels of IgE, and the lowest levels of sCD14 and, conversely, carriers of the _1,359G/_1,145G/_159T haplotype had the highest levels of sCD14 and the lowest IgE values (Vercelli *et al.*, 2001; Wang *et al.*, 2005).

The objectives of this investigation are to determine the prevalence of CD14 gene polymorphisms [C (-159)T, A(-1,145)G and G(-1,395)T] in asthmatic Saudi children patients and to determine the genotype as well as allele frequencies of these polymorphisms

Materials and Methods

Patients and collection of blood samples

A total of 42 childhood asthmatic patients diagnosed in the Prince Mansour Hospital, Taif, KSA were collected in the present work. Peripheral venous blood sample 3 ml was collected from asthma patients into EDTA anticoagulant Vacutainer tubes. Blood samples were stored in -20 ^{o}C until further steps.

DNA extraction

Genomic DNA was extracted from peripheral venous blood samples using blood gnomic DNA extraction kit according to manufacture structures of (Jena Bioscience: Jena Germany). Spectrophotometric determination of DNA concentration at A260 was done according to Karcher (1995). Quality of isolated DNA was checked by 1% agarose gel electrophoresis analysis. DNA samples were stored at –80 ^{o}C.

CD14 promoter amplification

The amplification of CD14 promoter was carried out via the PCR primers previously

described by Tan *et al.* (2006). During the present study, these primers were synthesized by Macrogen (Korea). PCR were performed in a reaction volume of 25 µl containing 50-100 ng DNA sample, 10 pmol of each primer and 12.5 µl of 2x SuperHot PCR Master Mix (Bioron, Ludwigshafen, Germany). The primers sequences and amplified PCR product of each studied polymorphism were summarized in table 1.

Thermal cycler program was 94C for 10 min, 35 cycles of 94C for 1 min, 65C for 1 min, 72 ^{o}C for 1 min, and a final extension of 72 ^{o}C for 10 min. All agarose gels were visualized and documented using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene; Frederick, Maryland, USA). The digital image files were analyzed using Gene Tools software from Syngene.

CD14 promoter genotyping

Following the PCR amplification each product was digested by specific restriction enzyme. The 295 bp amplified fragment of C (_159) T was digested by 2 U HaeIII (New England Biolabs, Frankfurt am Main, Germany). While, the fragment of A (1,145) G (371bp) was digested by HpyCH4 V (New England Biolabs). The polymorphism G (_1,359) T was genotyped by FokI (New England Biolabs) according to the manufacture instructions.

Specific restriction enzyme 10X buffer in reaction volume of 20 ul was used, incubation temperature and period as well as heat inactivation protocol were optimized and carried out. The digested DNA fragments were electrophoresed on 2% agarose gel stained with ethidium bromide. The gels were visualized on a UV Transilluminator, photographed using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene; Frederick, Maryland, USA).The digital image files were analyzed using Gene Tools software from Syngene.

Results and Discussion

In the present study three polymorphisms of CD14 gene were investigated. These polymorphisms are C (_159) T, A (_1,145) G and G (_1,359) T. The genotyping of the studied polymorphisms C (_159) T, A (_1,145) G and G (_1,359) T were detected Via PCR-RFLP assay with HaeIII, HpyCH4 and FokI restriction enzymes, respectively. The PCR product size of C(_159)T, A(_1,145)G and G(_1,359)T were 295, 371, 371 bp. The electrophoretic banding pattern of amplified fragments of each studied polymorphism was illustrated in figures 1-3.

Three genotypes were detected for C(_159)T polymorphism. The genotype distribution was CC (21.4%), CT (50%) and TT(28.6). the allele frequency was C(0.46) and T (0.54). The frequencies of observed genotypes for A(_1,145)G were AA(19 %), AG(45.2%) and GG(35.7%). The frequency of A allele was 0.42 and for G allele was 0.58. The resulted genotypes of G(_1,359)T polymorphism were GG, GT and TT. The % of these genotypes was 26.2, 42.8 and 31, respectively. The frequency of G and T alleles were allele 0.48 and 0.52.

Asthma caused by genetic and environmental factors and many genes have been identified in its pathogenesis (Sandford *et al.*, 1996). Twins studies have shown that, number of genes and their polymorphisms influence the immune and pulmonary development and response to the environmental factors, contributing to asthma occurrence and /or severity (Sarafino and Goldfedder, 1995; de Faria *et al.*, 2008).

Several reports have been reported that, there are different polymorphisms that have been described in the promoter region of

CD14 gene and has been associated with increased CD14 expression in vitro (LeVan *et al.,* 2001) and in the serum of children (Baldini *et al.,* 1999; Leung *et al.,* 2003) and with altered serum IgE levels and skin test positivity in different populations (Baldini *et al.,* 1999; Gao *et al.,* 1999; Ober *et al.,* 2000; Buckova *et al.,* 2003; Koppelman *et al.,* 2001; Leung *et al.,* 2003; O'Donnell *et al.,* 2003; Vercelli *et al.,* 2001).

Upon binding, CD14 signals enhance production of interleukin (IL) 12, which is in turn required for maturation of native T cells into type 1 helper T cells (TH1) cells, down-regulation of TH2 cells, and subsequent decreased immunoglobulin (Ig) E production (Holgate 1999; Micheal *et al.,* 2011).

Table.1 PCR primers used to detect CD14 gene polymorphisms

Polymorphism	Primer sequence	PCR product size
C(_159)T	5-ATCATCCTTTTCCCACACC-3 5-AACTCTTCGGCTGCCTCT-3	295 bp
A(_1,145)G	5-CTCAGGAATCTGAGGCAAGA-3 5-AGTACAATCTCTGTGCCCTA-3	371 bp
G(_1,359)T	5-CTCAGGAATCTGAGGCAAGA-3 5-AGTACAATCTCTGTGCCCTA-3	371 bp

Table.2 Prevalence% and allele frequencies of studied polymorphisms

Polymorphism	No of subjects	Genotypes			Allele frequency	
C(_159)T	42	CC	CT	TT	C	T
		9 (21.4%)	21 (50%)	12 (28.6%)	0.46	0.54
A(_1,145)G	42	AA	AG	GG	A	G
		8 (19 %)	19 (45.2%)	15 (35.7%)	0.42	0.58
G(_1,359)T	42	GG	GT	TT	G	T
		11(26.2%)	18 (42.8 %)	13 (31%)	0.48	0.52

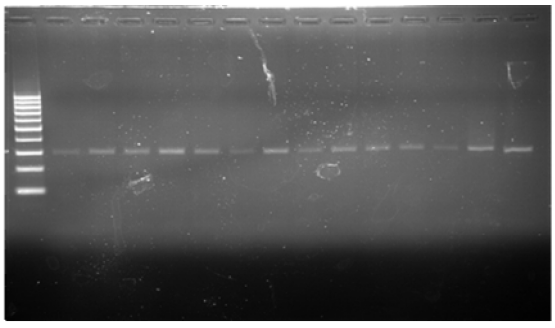

Fig.1: electrophoresis of PCR product (295bp) specific for CD14 promoter C(_159)T polymorphism among childhood asthma patients

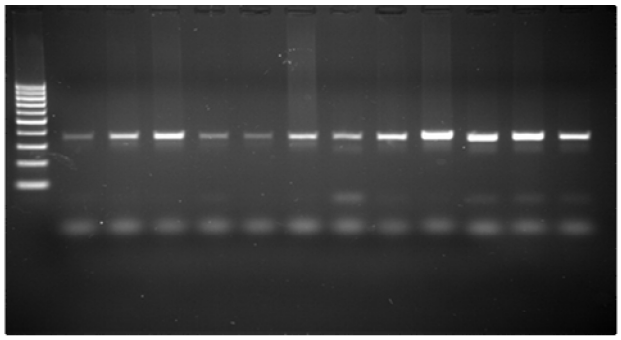

Fig.2: Electrophoresis of PCR product (370bp) specific for CD14 promoter A(_1,145)G polymorphism in childhood asthma patients

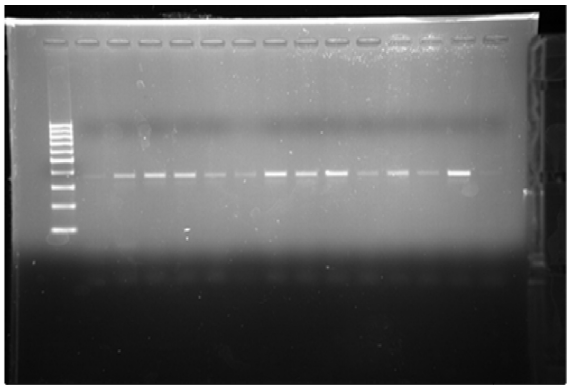

Fig.3: Electrophoresis of PCR product (370bp) specific for CD14 promoter G(_1,359)T polymorphism among childhood asthma patients

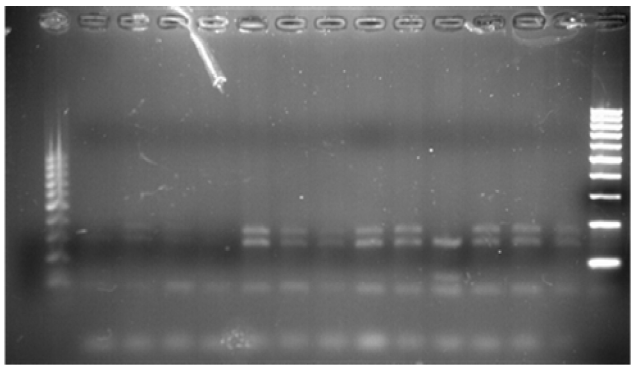

Fig.4 PCR product of promoter G(_1,359)T after digestion by FokI restriction enzyme

In the present study, three single nucleotide polymorphisms (SNPs), C(_159)T, A(_1,145)G, and G(_1,359)T, which were in strong linkage disequilibrium, in the promoter region of the CD14 gene were genotyped in Saudi asthmatic children using PCR-RFLP assay. Forty two childhood asthmatic patients were genotyped for C(_159)T, A(_1,145)G, and G(_1,359)T polymorphisms. The prevalence percentage of C(_159)T genotypes (CC, CT and TT) was (21.4, 50 and 28.6) the allele frequencies were 0.46 for C and 0.54 for T.

The analysis of A(_1,145)G revealed that the prevalence of observed genotypes (AA, AG and GG) was (19, 45.2 and 35.7) and the frequency of A allele was 0.42 and 0.58 for G allele.

The G(_1,359)T polymorphism manifested (GG, GT and TT) genotypes with prevalence % (26.2, 42.8 and 31). The G allele frequency was 0.48 and 0.52 for T allele. These findings are inconsistent with different studies among other populations. de Faria *et al.* (2008) suggested significant association between TT genotype and severe asthma. Moreover, they reported that the C(_159)T polymorphism might be involved in modulation of asthma severity. Another study conducted by Yazdani *et al.* (2012) reported that, patients with CC genotype at position -159 of the CD14 promoter region had an increase of asthma. Bisyuk (2014) demonstrated that, the CD14-C159T polymorphism is associated with adult non-atopic asthma but not with adult atopic asthma in the Crimean Ukraine population adults. Tan *et al.* (2006) reported that CD14 gen variants play an important role in influencing allergen sensitization of children in Taiwan. Woo *et al.* (2003) demonstrated that, the TT genotype of -159 C-->T CD14

is associated with nonatopic asthma and food allergy. Han *et al.* (2010) stated that, the TT homozygotes are more common in adult patients with allergic rhinitis among the Chinese population. In Pakistani population Micheal *et al.* (2001) studied the association between some CD14 polymorphisms asthma among asthmatic patients. The results indicated that, the A-1145G polymorphism is associated with atopic asthma, whereas the C-159T polymorphism is significantly associated with allergic rhinitis. Rennie *et al.* (2013) assessed the role of CD14 C-159T, G-1359T in the expression of asthma, croup, and allergy in Canadian school children of ages 6 to 14 years. Results obtained demonstrated that, Haplotype analyses of the two CD14 polymorphisms showed that individuals with the TT haplotype combination were significantly more likely to have asthma.

In the present study three polymorphisms of CD14 promoter gene were genotyped among 42 childhood asthmatic patients. We conclude that the heterogeneous genotypes CT, AG and GT were the highest prevalent genotypes among studied patients. Further and larger studies are needed to investigate the role of other polymorphisms in the etiology of childhood asthma.

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