The Journal of Basic & Applied Zoology (2013) **66**, 188–194

The Egyptian German Society for Zoology

The Journal of Basic & Applied Zoology

www.egsz.org
www.sciencedirect.com

Molecular genetic analysis of polymorphisms pertaining to the susceptibility to chronic asthma in Egyptian patients

Ahmed A.A. Ali [a,*], **Ahmad A. Settin** [b]

[a] *Zoology Dept., Faculty of Science, Sohag University, Egypt*
[b] *Department of Pediatric, Faculty of Medicine, Mansoura University, Egypt*

Received 9 March 2013; revised 16 May 2013; accepted 16 May 2013
Available online 12 June 2013

KEYWORDS

Asthma;
Cytokines;
Gene polymorphism;
IL-10;
IL-6;
TNF-α;
IL-1Ra

Abstract *Background:* Asthma is a multifactorial inflammatory disorder that might result from the interaction of multiple genes and environmental factors. Several studies of genetic epidemiology have reported the association of cytokine genes with asthma among various populations. However, the results are inconsistent and inconclusive.

Objective: To determine the association of cytokine gene polymorphisms with asthma susceptibility in adult Egyptian cases.

Subjects: The study included, 50 adult Egyptian asthmatic cases with a mean age of 53.62 ± 14.61 years in addition to 98 healthy individuals as control. For all participants, DNA was isolated from peripheral blood samples and analyzed for TNF-α-308 G > A, IL-10-1082 G > A, IL-6-174 G > C and IL-1Ra VNTR polymorphisms.

Results: The comparison between the cases and controls has showed significantly higher frequency of the genotypic polymorphisms: IL-10-1082 AG + GG (dominant mode) (76.1% vs. 91.8%, $p = 0.01$, OR = 0.3, 95% CI (0.1–0.7), TNF-α-308 GA + AA (dominant mode) (72.0% vs. 93.9%, $p = 0.001$, OR = 0.17, 95% CI = 0.06–0.47) and IL-1RA VNTR heterozygous genotype A1A2 (90.0% vs. 58.8%, $p = 0.0003$). Otherwise, compared to controls, cases showed

Abbreviations: TNF, tumor necrosis factor; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; SNP, single nucleotide polymorphism; VNTR, variable number tandem repeat; PCR–SSP, polymerase chain reaction with sequence specific primers

* Corresponding author. Address: Department of Zoology, College of Science, Sohag University, Egypt.
E-mail address: AAh592003@yahoo.com (A.A.A. Ali).

Peer review under responsibility of The Egyptian German Society for Zoology.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jobaz.2013.05.001

statistically non-significant frequency of genotypes corresponding to IL-6-174 CC + GC vs. GG (dominant mode) (92.0% vs. 94.9%, $p = 0.5$, OR = 0.6).

Conclusions: The IL-10, TNF-α and IL-1Ra allelic variants showed a potent association with chronic asthma among Egyptian cases that might be used as markers with a potential impact on prophylactic and or therapeutic measures for asthma control. Whereas the IL-6-174 allelic variants showed no association with chronic asthma among Egyptian cases.

Introduction

Asthma is one of the most common chronic airway inflammatory diseases characterized by reversible airway obstruction and by bronchial hyper-responsiveness (BHR) with periodic episodes of wheezing. Asthma and atopy have a complex background that may result from the interaction of genetic and environmental factors (Custovic et al., 2012). The control of asthma and response to medication is different in patients, and different asthmatics show various levels of asthma severity and progress depending on multiple factors especially genetic profile of the patients. The airway inflammatory response of asthma is now known to be associated with the release of a number of inflammatory mediators and cytokines. The genes that predispose individuals to asthma are not consistent among populations as one individual may have a cytokine expression pattern totally different from another (Michel et al., 2010). In fact, clinical symptoms of asthma may reflect an imbalance in pro- and anti-inflammatory cytokine levels (Choi et al., 2008). However, cytokines play a pivotal role in the regulation of immune response and the polymorphic structure of the regulatory regions in the promoters of cytokine genes could affect the serum levels of cytokines, consequently may confer flexibility on the immune response (Melk et al., 2003; Stanilova and Miteva, 2005).Therefore, a large number of studies have focused on the association between cytokine gene polymorphisms and asthma risk (Steinke et al., 2008; Himes et al., 2009).

Interleukin-10 is one of pleiotropic immunoregulatory cytokines formed by alveolar macrophages, T-regulatory cells, B cells, mast cells, monocytes, and pulmonary dendritic cells (Miteva and Stanilova, 2008). Alveolar macrophages in asthmatic patients were found to produce significantly less amount of IL-10 (Zhang et al., 2002). The gene for IL-10 is located on chromosome 1q31–32, a genomic region linked to asthma and related phenotypes (Yu et al., 2004). A number of polymorphisms in the promoter region of IL-10 gene have been described; in particular, the SNP at position −1082A/G of noting that IL-10A allele was reported to be associated with lower production of IL-10 (Moore, 2001). Several studies examined the association of IL-10 polymorphisms with allergic diseases. However, the results have been inconsistent (Karjalainen et al., 2003; Chatterjee et al., 2005; Sohn et al., 2007; Kim et al., 2009).

IL-6 is another immunoregulatory pleiotropic cytokine that plays a role in inflammation of asthma (Córdoba-Lanús, 2008; Rincon and Irvin, 2012) and increased levels of IL-6 in serum have been found in asthmatic patients (Yokoyama et al., 1995). More importantly, a study examining IL-6 in the brochoalveolar lavage fluid (BALF) has shown increased levels of IL-6 in active asthmatic patients compared with the levels in healthy nonsmoker subjects, stable asthmatic and non-asthmatic patients receiving mechanical ventilation (Tillie-Leblond et al., 1999). In addition, increased levels of IL-6 in the BALF from patients with "intrinsic" asthma compared with the levels in patients with allergic asthma have also been reported Virchow et al., 1996), suggesting that IL-6 may play a role beyond patients with allergic asthma which only accounts for about 50% of all asthmatics. The gene encoding IL-6 was mapped to chromosome 7p21 (Yalçın et al., 2011). A number of polymorphisms in the IL-6 gene promoter region exhibited to be related to cytokine plasmatic levels. Most of the studies confirmed that IL-6-174 G/C was associated with different inflammatory diseases (Fishman et al., 1998). However, the GG and GC genotypes led to a high production of IL 6, while, CC genotype led to a low production of this cytokine (Morse et al., 1999). Consequently, this variant is associated with reduced pulmonary capacities (Daneshmandi et al., 2012).

Tumor necrosis factor-α (TNF-α) is a potent proinflammatory cytokine that participates in the airway inflammatory response in cases with atopic asthma. However, pervious study reported that the TNF-α plays a key role in the pathogenesis of bronchial asthma (BA) among various populations and increased bronchial hyper responsiveness (Gao et al., 2006; Daneshmandi et al., 2012). TNF-α gene is located in the human major histocompatibility complex (MHC) on chromosome 6p21.3 and its expression is increased in cases of severe asthma (Ying et al., 1991). This site was previously linked with asthma susceptibility and genetic studies have identified associations between TNF-α gene polymorphisms. Indeed, TNF-α-308 promoter polymorphism is biallelic, G and A noting that the A allele was reported to be associated with increased levels of TNF-α in the plasma and bronchoalveolar lavage fluid from asthmatic airways (Choi, 2005). Also, it was reported to be associated with increased in vitro transcription of TNF-α and TNF-α level in the stimulated human white blood cells (Lee et al., 2009). Recent meta-analysis indicated that the TNF-α-308 G/A polymorphism is associated with an increased risk of asthma in adults and children, in Asians but not in Caucasians, and in atopic population but not in non-atopic population (Zhang et al., 2011).

IL-1Ra has anti-inflammatory activity and thereby reduces airway responsiveness due to the initially secreted proinflammatory cytokines (Mao et al., 2000). The IL-1 cluster, mapped on chromosome 2q12–14, harbors the IL-1Ra gene that contains an 86 bp VNTR polymorphism in intron 2 (Tarlow et al., 1993; Hakonarson and Wjst, 2001) resulting into five different alleles with numbers of repeats ranging between 2 and 6. The two most common alleles, allele 1 and 2, account for approximately 98% of all observed alleles. In vitro and in vivo studies investigating the level of IL-1Ra have found IL-1Ra alleles to influence the levels of the expressed protein (Ahirwar et al., 2008).

In this study, we investigated the associations of the TNF-α-308G/A, IL-6-174G/C, IL-10 1082G/A and IL-Ra

VNTR polymorphisms with respect to chronic asthma susceptibility in adult Egyptian patients.

Subjects and methods

Participants composed of 50 adult chronic asthmatic cases who were followed up in the Clinics of College of Medicine, Mansoura University. They included 22 (44%) males and 28 (56%) females with an age mean of 53.62 ± 14.61 years. Of these cases, 15 (30%) had a positive parental consanguinity and 24 (48%) had a positive family history of asthma. Asthma diagnosis was based on the international consensus reports on diagnosis and treatment of asthma and physicians' recommendations (National Heart, Lung and Blood Institute, 2007). Polymorphisms of cytokine genes of cases were compared with those of 98 healthy unrelated volunteers from the same locality. They included 52 males and 46 females with a mean age of 44.9 ± 6.7 years.

It is worth mentioning that all the volunteers and the cases have already agreed to use their samples in genetic examination.

DNA isolation and PCR

Genomic DNA was extracted from fresh peripheral blood (3 ml in EDTA) using a commercially available kit (Qiagen, Germany) according to the manufacturer's instruction and then stored at −20 °C till use. Single nucleotide polymorphisms (SNPs) related to the TNF-α (-308), IL-6 (-174) and IL-10 (-1082) were determined using PCR with sequence-specific primers (PCR–SSP) in two reactions employing one common forward and two reverse primers; while IL-1Ra VNTR polymorphism was determined by a single PCR reaction employing one forward and one reverse primer. The reaction mix was done in 25 μl volumes of: 100 ng of template DNA added to a mix of 1 μM of each common/specific primer, 1 mM $MgCl_2$, 200 μM of each dNTP, and 1 U of Ampli Taq polymerase (Applied Biosystems, USA). In each PCR run, a heterozygous control template was used to ensure accuracy. The sequences of designed primers and PCR conditions for each gene are shown in Table 1. The resultant PCR products were resolved by electrophoresis on 2% agarose gel stained with ethidium bromide for 20 min at 200 V. The gel was then photographed on UV light (320 nm) and scored for the presence or absence of an allele specific band (Fig. 1).

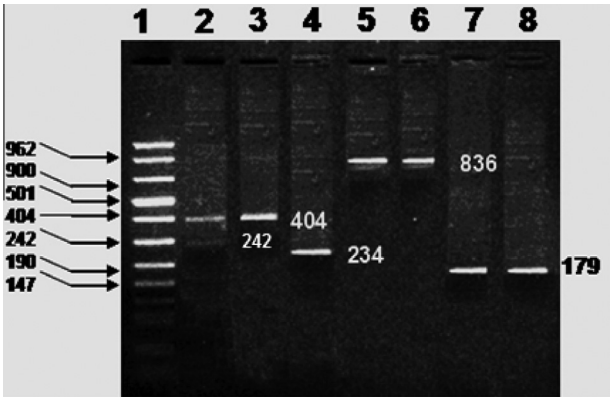

Figure 1 Amplification products. Lane 1: size marker VIII. Lanes 2 and 3: amplified product of IL1 Ra (VNTR) with 2 alleles of size 404, 242 bp (lane 2) and one allele of size 404 bp (lane 3). Lane 4: amplified product of IL-6 shows a band size of 234 bp. Lanes 5 and 6: amplified product of TNF-α band size 836 bp. Lanes 7 and 8: amplified product of IL-10 band size 179 bp.

Statistical analysis

Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 17.0). The frequency of studied allelic polymorphisms among cases was compared to that of controls and tested for positive association using Fisher's exact test and Odds ratio with a minimum level of significance of <0.05.

Results

Comparing the genotypic variants of cases to controls pertaining their IL-10-1082 A > G polymorphism, it was noticed that the frequency of GG + GA vs. AA (dominant mode) was significantly lower among cases than controls (76.1% vs. 91.8%, $p = 0.01$, OR = 0.3, 95% CI (0.1–0.7)). In the meantime, the frequency of GA vs. AA (additive mode) was significantly higher among cases than controls (86% vs. 66.0%, $P = 0.009$, OR 0.3, 95% CI (0.1–0.7)). Otherwise, compared to controls, cases showed statistically non-significant frequency of genotypes corresponding to IL-6-174 G > C polymorphism-CC + GC vs. GG, dominant mode – (92.0% vs. 94.9%, $p = 0.5$, OR = 0.6 (0.16–2.4)). Whereas, comparing

Table 1 Primer sequences and PCR conditions of the different studied cytokine genes.

Gene	Primer sequences 5'–3'	PCR product size (bp)	PCR conditions
IL-10 1082G/A	F: AGC AAC ACT CCT CGT CGC AAC R1: CCT ATC CCT ACT TCC CCC R2: CCT ATC CCT ACT TCC CCT	179	30 cycles: 94 °C (30 s), 60 °C (60 s), 72 °C (60 s) then 72 °C (7 min)
IL-6-174G/C	F: GAG CTT CTC TTT CGT TCC R1: CCT AGT TGT GTC TTG CC R2: CCC TAG TTG TGT CTT GCG	234	30 cycles: 94 °C (30 s), 54 °C (60 s), 72 °C (60 s) then 72 °C (7 min)
TNF-α-308G/A	F: CTG CAT CCC CGT CTT TCT CC R1: ATA GGT TTT GAG GGG CAT CG R2: ATA GGT TTT GAG GGG CAT CA	836	30 cycles: 96 °C (45 s), 55 °C (80 s) and 72 °C (2 min), then 72 °C (3 min)
IL-1Ra VNTR	F: TCC TGG TCT GCA GGT AA R: CTC AGC AAC ACT CCT AT	404–242	35 cycles: 94 °C (1 min), 60 °C (1 min), 70 °C (1 min), then 70 °C (5 min)

Table 2 Frequency of IL-10, IL-6, TNF-α and IL-1Ra genotype polymorphism among adult Egyptian asthmatic patients compared to controls.

Genotypes	Cases *n* (%)	Control n (%)	Fisher (P)	OR	95% CI
IL-10-1082 A > G					
Recessive mode GG vs. AA + GA	5 (10.1) vs. 45 (89.9)	5 (5.1) vs. 93 (94.9)	0.3	2.1	0.6–7.5
Dominant mode GG + GA vs. AA	38 (76.1) vs. 12 (24.0)	90 (91.8) vs. 8 (8.2)	0.01*	0.3	0.1–0.7
Additive mode GA vs. AA	33(66.0) vs. 12(24.0)	85(86.0) vs. 8(8.2)	0.009*	0.3	0.1–0.7
Additive mode GA vs. GG	33(66.0) vs. 5(10.1)	85(86.0)vs. 5(5.1)	0.15	0.4	0.1–1.4
IL-6-174 G > C					
Recessive mode CC vs. GG + GC	3(6.0)vs. 47(94.0)	6(6.1)vs. 92(93.9)	1.000	0.978	0.23–4.09
Dominant mode CC + GC vs. GG	46(92.0) vs.4(8.0)	93(94.9) vs. (5(5.1)	0.5	0.62	0.16–2.4
Additive mode GC vs. GG	43(86.0) vs. 4(8.0)	87(88.8) vs. 5(5.1)	0.5	0.62	0.16–2.4
Additive mode GC vs. CC	43(86.0) vs. 3(6.0)	87(88.8) vs. 6(6.1)	1.0	0.99	0.24–4.14
TNF-α-308 G > A					
Recessive mode AA vs. GG + GA	7(14.0) vs. 43(86)	11(11.2) vs.87(88.8)	0.6	1.3	0.47–3.6
Dominant mode AA + GA vs. GG	36(72.0) vs. 14(28.0)	92(93.9) vs. 6(6.1)	0.001**	0.17	0.06–0.47
Additive mode GA vs. AA	29(58.0) vs. 7(14.0)	81(82.7) vs. 11(11.2)	0.28	0.6	0.2–1.6
Additive mode GA vs. GG	29(58.0) vs. 14(28.0)	81(82.7) vs. 6(6.1)	0.0004**	0.15	0.05–0.44
IL-1RA VNTR					
Dominant mode A1A1 vs. A1A2	45(90.0) vs. 5(10.0)	57(58.8) vs. 40(41.2)	0.0003**	6.31	2.30–17.31
Additive mode A1A2 vs. A1A1	5(10.0) vs. 45(90.0)	40(41.2) vs. 57(58.8)	0.0003**	0.15	0.05–0.43

* Significant at $P < 0.05$.
** Significant at $P < 0.001$.

the genotypic variants of cases to controls pertaining their TNF-α-308 G > A polymorphism, it was noticed that the frequency of AA + GA vs. GG (dominant mode) was significantly higher among cases than controls (72.0% vs. 93.9%, $p = 0.001$, OR = 0.17, 95% CI (0.06–0.47)). While, the frequency of GA vs. GG (additive mode) was highly significant among cases than controls (58.0% vs. 82.7%, $P = 0.0004$, OR 0.15, 95% CI (0.05–0.44)). Regarding to the frequency of genotypic variants related to IL-1RA VNTR polymorphism, it was noticed that the frequency of A1A1 vs. A1A2 (dominant mode) was significantly higher among cases than controls (90.0% vs. 58.8%, $p = 0.0003$, OR = 6.31, 95% CI (2.3–17.3)). The frequency of A1A2 vs. A1A1 (additive mode) was highly significant among cases than controls (10.0% vs. 41.2%, $P = 0.0003$, OR 0.15, 95% CI (0.05–0.43)) (Table 2).

Discussion

Gene-by-environmental factor seems to be a key process in the development and expression of asthma (Choi et al., 2008). The airway inflammatory component of asthma is partly controlled by the genetic background of the patients (Holgate et al., 1995). Whereas, several linkage and association studies have suggested the presence of various candidate atopy and asthma genes, it is difficult to detect consistent abnormalities in these genes that may have disease-modifying effects (Wjst et al., 1999).

Most association studies of polymorphisms in the promoter region of IL-10 and asthma-related phenotypes have been positive. Although these polymorphisms have been shown to influence levels of IL-10 expression, there have been conflicting findings with regard to the direction of these associations (Movahedi et al., 2008). Our current study showed a significant association between IL-10-1082 AA genotypic polymorphism (dominant mode) and asthma in adult Egyptian patients. This result is in agreement with the finding of Trajkov et al. in Macedonian population (Trajkov et al., 2008). In addition this present study showed a significant association between IL-10-1082 GA genotypic polymorphism (additive mode) and asthma in adult Egyptian patients. Also, in a previous study done on asthmatic Egyptian children, Zedan et al. reported that IL-10-1082GA genotypes may be contributing factors to the susceptibility and severity of childhood asthma (Zedan et al., 2008). IL-10-1082GA polymorphism was also confirmed to be associated with asthma in Iranian population (Movahedi et al., 2008), North Indians (Kim et al., 2008) and in Chinese patients (Zhang et al., 2002). On the other hand, IL-10 polymorphism was not found to be related to susceptibility of asthma in adult Finnish population (Karjalainen et al., 2003).

Analysis of genetic polymorphism of IL-6-174 in asthmatic Egyptian patients revealed a non-significant association, however, in a previous study; researchers reported a significant association between IL-6-174 GG and asthma in Egyptian children (Settin et al., 2008). This same association was found positive in another study conducted on Iranian subjects with asthma (Mahdaviani et al., 2009). The difference in genetic polymorphisms associated with childhood and adult asthma possibly points to the different pathophysiological mechanisms underlying both entities which also coincide with the different presentations of both types of asthma.

Results of the current study showed that TNF-α-308 GG (dominant mode) polymorphism is significantly associated with chronic asthma in adult Egyptian patients. It was previously reported that TNF-α-308 GA genotypic polymorphism may be a contributing factor to the susceptibility and severity of asthma in Egyptian children (Zedan et al., 2008). A similar finding was reported by Witte et al. (2002) that TNF-α-308 GG was positively associated with asthma in Californian patients. Previous association studies concluded that TNF-α-308 GA promoter polymorphism was significantly associated with asthma in North Indian patients (Choi, 2005; Gupta et al., 2005; Gao et al., 2006), Punjabi population (Kumar

et al., 2008), Iranian population (Movahedi et al., 2008) and in Korean children (Kim et al., 2008). Another study of G-308A TNF-α polymorphism on children from Western Australia revealed that the G allele was present at higher frequency in asthmatics as compared to controls (Albuquerque et al., 1998). On the contrary, other investigators failed to find an association between G-308A polymorphic alleles and asthma among: Turkish population (Bucková et al., 2002; Aytekin et al., 2009) and American population (El Bahlawan et al., 2003).

The polymorphism of IL-1 receptor antagonist (IL-1Ra) gene has been previously investigated in different populations with asthma (Gohlke et al., 2004; Pattaro et al., 2006). Analysis of IL-1Ra VNTR polymorphism in adult Egyptian patients with asthma showed the presence of high significant frequency of the homozygous genotype forms A1/A1 (dominant mode) and A1A2 (additive mode). This finding is matched with a previous study conducted on Egyptian children with asthma (Settin et al., 2008). The same finding was also reported in a previous study among Turkish children with asthma (Zeyrek et al., 2008). Analysis of allelic polymorphism of all studied genes revealed only IL-1Ra A1 allele that showed significant higher frequency among chronic adult asthmatic cases compared to controls among Egyptian population. Pillay et al. (2000) studied different allelic polymorphisms of IL-Ra and concluded that the IL-1 Ra is a marker of disease severity in asthma, particularly in black patients.

From this study, it is concluded that cytokine gene polymorphisms showed a positive association with chronic asthma that might suggest their usage as markers for asthma among Egyptian families with a potential impact on prophylactic and or therapeutic measures for asthma control. However we recommend further longitudinal study with a larger sample size and familial cases for further association and linkage analyses.

References

Ahirwar, D., Kesarwani, P., Manchanda, P.K., Mandhani, A., Mittal, R.D., 2008. Anti- and proinflammatory cytokine gene polymorphism and genetic predisposition: association with smoking, tumor stage and grade, and bacillus Calmette-Guérin immunotherapy in bladder cancer. Cancer Genet. Cytogenet. 184 (1), 1–8.

Albuquerque, R.V., Hayden, C.M., Palmer, L.J., Laing, I.A., Rye, P.J., Gibson, N.A., Burton, P.R., Goldblatt, J., Lesouëf, P.N., 1998. Association of polymorphisms within the tumour necrosis factor (TNF) genes and childhood asthma. Clin. Exp. Allergy 28 (5), 578–584.

Aytekin, C., Doğu, F., Ikincioğullari, A., Eğin, Y., Yüksek, M., Bozdoğan, G., Akar, N., Babacan, E., 2009. TGF-Beta1-915G/C and TNF-α-308G/A polymorphisms in children with asthma. Tuberk Toraks 57 (1), 62–67.

Bucková, D., Hollá, L.I., Vasků, A., Znojil, V., Vácha, J., 2002. Lack of association between atopic asthma and the tumor necrosis factor alpha-308 gene polymorphism in a Czech population. J. Investig. Allergol. Clin. Immunol. 12 (3), 192–197.

Chatterjee, R., Batra, J., Kumar, A., Mabalirajan, U., Nahid, S., Niphadkar, P.V., Ghosh, B., 2005. Interleukin-10 promoter polymorphisms and atopic asthma in North Indians. Clin. Exp. Allergy 35 (7), 914–919 (PubMed PMID: 16008678).

Choi, I.W., Sun-Kim, Kim, Y.S., Ko, H.M., Im, S.Y., Kim, J.H., You, H.J., Lee, Y.C., Lee, J.H., Park, Y.M., Lee, H.K., 2005. TNF-alpha induces the late-phase airway hyper responsiveness and airway inflammation through cytosolic phospholipase A(2) activation. J. Allergy Clin. Immunol. 116 (3), 537–543.

Choi, I.S., Cui, Y., Koh, Y.A., Lee, H.C., Cho, Y.B., Won, Y.H., 2008. Effects of dehydroepiandrosterone on Th2 cytokine production in peripheral blood mononuclear cells from asthmatics. Korean J. Intern. Med. 23 (4), 176–181.

Córdoba-Lanús, E., De-Torres, J.P., López-Aguilar, C., Rodríguez-Pérez, M.C., Maca-Meyer, N., Montejo-de-Garcini, A., Aguirre-Jaime, A., Pérez-Méndez, L., Casanova, C., 2008. Association of IL-6 gene polymorphisms and COPD in a Spanish population. Respir. Med. 102 (12), 1805–1811.

Custovic, A., Marinho, S., Simpson, A., 2012. Gene–environment interactions in the development of asthma and atopy. Expert Rev. Respir. Med. 6 (3), 301–308. http://dx.doi.org/10.1586/ers.12.24 (Review. PubMed PMID: 22788944).

Daneshmandi, S., Pourfathollah, A.A., Pourpak, Z., Heidarnazhad, H., Kalvanagh, P.A., 2012. Cytokine gene polymorphism and asthma susceptibility, progress and control level. Mol. Biol. Rep. 39 (2), 1845–1853. http://dx.doi.org/10.1007/s11033-011-0927-7 (Epub 2011 Jun 3. PubMed PMID: 21637953).

El Bahlawan, L., Christensen, M., Binaei, S., Murphy, C., Zhang, Q., Quasney, M., 2003. Lack of association between the tumor necrosis factor-alpha regulatory region genetic polymorphisms associated with elevated tumor necrosis factor-alpha levels and children with asthma. Chest 123 (3), 374S–375S.

Fishman, D., Faulds, G., Jeffery, R., Mohamed-Ali, V., Yudkin, J.S., Humphries, S., Woo, P., 1998. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J. Clin. Invest. 102 (7), 1369–1376.

Gao, J., Shan, G., Sun, B., Thompson, P.J., Gao, X., 2006. Association between polymorphism of tumour necrosis factor alpha-308 gene promoter and asthma: a meta-analysis. Thorax 61 (6), 466–471.

Gohlke, H., Illig, T., Bahnweg, M., Klopp, N., André, E., Altmüller, J., Herbon, N., Werner, M., Knapp, M., Pescollderungg, L., Boner, A., Malerba, G., Pignatti, P.F., Wjst, M., 2004. Association of the interleukin-1 receptor antagonist gene with asthma. Am. J. Respir. Crit. Care Med. 169 (11), 1217–1223.

Gupta, V., Sarin, B.C., Changotra, H., Sehajpal, P.K., 2005. Association of G-308A TNF-alpha polymorphism with bronchial asthma in a North Indian population. J. Asthma 42 (10), 839–841.

Hakonarson, H., Wjst, M., 2001. Current concepts on the genetics of asthma. Curr. Opin. Pediatr. 13 (3), 267–277.

Himes, B.E., Hunninghake, G.M., Baurley, J.W., Rafaels, N.M., Sleiman, P., Strachan, D.P., Wilk, J.B., Willis-Owen, S.A., Klanderman, B., Lasky-Su, J., Lazarus, R., Murphy, A.J., Soto-Quiros, M.E., Avila, L., Beaty, T., Mathias, R.A., Ruczinski, I., Barnes, K.C., Celedón, J.C., Cookson, W.O., Gauderman, W.J., Gilliland, F.D., Hakonarson, H., Lange, C., Moffatt, M.F., O'Connor, G.T., Raby, B.A., Silverman, E.K., Weiss, S.T., 2009. Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. Am. J. Hum. Genet. 84 (5), 581–593. http://dx.doi.org/10.1016/j.ajhg.2009.04.006 (Epub 2009 May 7. PubMed PMID: 19426955; PubMed Central PMCID: PMC2681010).

Holgate, S.T., Church, M.K., Howarth, P.H., Morton, E.N., Frew, A.J., Djukanović, R., 1995. Genetic and environmental influences on airway inflammation in asthma. Int. Arch. Allergy Immunol. 107 (1–3), 29–33.

Karjalainen, J., Hulkkonen, J., Nieminen, M.M., Huhtala, H., Aromaa, A., Klaukka, T., Hurme, M., 2003. Interleukin-10 gene promoter region polymorphism is associated with eosinophil count and circulating immunoglobulin E in adult asthma. Clin. Exp. Allergy 33 (1), 78–83 (PubMed PMID: 12534553).

Kim, H.B., Kang, M.J., Lee, S.Y., Jin, H.S., Kim, J.H., Kim, B.S., Jang, S.O., Lee, Y.C., Sohn, M.H., Kim, K.E., Hong, S.J., 2008. Combined effect of tumour necrosis factor-alpha and interleukin-13 polymorphisms on bronchial hyperresponsiveness in Korean children with asthma. Clin. Exp. Allergy 38 (5), 774–780.

Kim, S.H., Yang, E.M., Lee, H.N., Cho, B.Y., Ye, Y.M., Park, H.S., 2009. Combined effect of IL-10 and TGF-beta1 promoter polymorphisms as a risk factor for aspirin-intolerant asthma and rhinosinusitis. Allergy 64 (8), 1221–1225. http://dx.doi.org/10.1111/j.1398-9995.2009.01989.x (Epub 2009 Feb 16. PubMed PMID: 19222424).

Kumar, A., Gupta, V., Changotra, H., Sarin, B.C., Sehajpal, P.K., 2008. Tumor necrosis factor-alpha and transforming growth factor-beta1 polymorphisms in bronchial asthma. Indian J. Med. Sci. 62 (8), 323–330.

Lee, Y.L., McConnell, R., Berhane, K., Gilliland, F.D., 2009. Ambient ozone modifies the effect of tumor necrosis factor G-308A on bronchitic symptoms among children with asthma. Allergy 64 (9), 1342–1348. http://dx.doi.org/10.1111/j.1398-9995.2009.02014.x, Epub 2009 Feb 16. PubMed PMID: 19236316.

Mahdaviani, S.A., Rezaei, N., Moradi, B., Dorkhosh, S., Amirzargar, A.A., Movahedi, M., 2009. Proinflammatory cytokine gene polymorphisms among Iranian patients with asthma. J. Clin. Immunol. 29 (1), 57–62.

Mao, X.Q., Kawai, M., Yamashita, T., Enomoto, T., Dake, Y., Sasaki, S., Kataoka, Y., Fukuzumi, T., Endo, K., Sano, H., Aoki, T., Kurimoto, F., Adra, C.N., Shirakawa, T., Hopkin, J.M., 2000. Imbalance production between interleukin-1beta (IL-1beta) and IL-1 receptor antagonist (IL-1Ra) in bronchial asthma. Biochem. Biophys. Res. Commun. 276 (2), 607–612.

Melk, A., Henne, T., Kollmar, T., Strehlau, J., Latta, K., Offner, G., Jhangri, G.S., Ehrich, J.H., Von Schnakenburg, C., 2003. Cytokine single nucleotide polymorphisms and intrarenal gene expression in chronic allograft nephropathy in children. Kidney Int. 64 (1), 314–320.

Michel, S., Liang, L., Depner, M., Klopp, N., Ruether, A., Kumar, A., Schedel, M., Vogelberg, C., Von Mutius, E., Von Berg, A., Bufe, A., Rietschel, E., Heinzmann, A., Laub, O., Simma, B., Frischer, T., Genuneit, J., Gut, I.G., Schreiber, S., Lathrop, M., Illig, T., Kabesch, M., 2010. Unifying candidate gene and GWAS approaches in asthma. PLoS One 5 (11), e13894. http://dx.doi.org/10.1371/journal.pone.0013894, PubMed PMID: 21103062; PubMed Central PMCID: PMC2980484.

Miteva, L., Stanilova, S., 2008. The combined effect of interleukin (IL)-10 and IL-12 polymorphisms on induced cytokine production. Hum. Immunol. 69 (9), 562–566.

Moore, K.W., De Waal Malefyt, R., Coffman, R.L., O'Garra, A., 2001. Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. 19, 683–765.

Morse, H.R., Olomolaiye, O.O., Wood, N.A., Keen, L.J., Bidwell, J.L., 1999. Induced heteroduplex genotyping of TNF-alpha, IL-1beta, IL-6 and IL-10 polymorphisms associated with transcriptional regulation. Cytokine 11 (10), 789–795.

Movahedi, M., Mahdaviani, S.A., Rezaei, N., Moradi, B., Dorkhosh, S., Amirzargar, A.A., 2008. IL-10, TGF-beta, IL-2, IL-12, and IFN-gamma cytokine gene polymorphisms in asthma. J. Asthma 45 (9), 790–794.

National Heart, Lung and Blood Institute, 2007. Expert Panel Report 3 (EPR3): Guidelines for the Diagnosis and Management of Asthma. NIH.

Pattaro, C., Heinrich, J., Werner, M., de Marco, R., Wjst, M., 2006. Association between interleukin-1 receptor antagonist gene and asthma-related traits in a German adult population. Allergy 61 (2), 239–244.

Pillay, V., Gaillard, M.C., Halkas, A., Song, E., Dewar, J.B., 2000. Differences in the genotypes and plasma concentrations of the interleukin-1 receptor antagonist in black and white South African asthmatics and control subjects. Cytokine 12 (6), 819–821 (PubMed PMID: 10843772).

Rincon, M., Irvin, C.G., 2012. Role of IL-6 in asthma and other inflammatory pulmonary diseases. Int. J. Biol. Sci. 8 (9), 1281–1290. http://dx.doi.org/10.7150/ijbs.4874 (Epub 2012 Oct 25. Review. PubMed PMID: 23136556; PubMed Central PMCID: PMC3491451).

Settin, A., Zedan, M., Farag, M., Ezz El Regal, M., Osman, E., 2008. Gene polymorphisms of IL-6(-174) G/C and IL-1Ra VNTR in asthmatic children. Indian J. Pediatr. 75 (10), 1019–1023.

Sohn, M.H., Song, J.S., Kim, K.W., Kim, E.S., Kim, K.E., Lee, J.M., 2007. Association of interleukin-10 gene promoter polymorphism in children with atopic dermatitis. J. Pediatr. 150 (1), 106–108 (PubMed PMID: 17188628).

Stanilova, S., Miteva, L., 2005. Taq-I polymorphism in 3′UTR of the IL-12B and association with IL-12p40 production from human PBMC. Genes Immun. 6 (4), 364–366 (PubMed PMID: 15858599).

Steinke, J.W., Rich, S.S., Borish, L., 2008. 5 Genetics of allergic disease. J. Allergy Clin. Immunol. 121 (2 Suppl.), S384–S387. http://dx.doi.org/10.1016/j.jaci.2007.07.029 (quiz S416. Review. PubMed PMID: 18241687).

Tarlow, J.K., Blakemore, A.I., Lennard, A., Solari, R., Hughes, H.N., Steinkasserer, A., Duff, G.W., 1993. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum. Genet. 91 (4), 403–404.

Tillie-Leblond, I., Pugin, J., Marquette, C.H., Lamblin, C., Saulnier, F., Brichet, A., Wallaert, B., Tonnel, A.B., Gosset, P., 1999. Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. Am. J. Respir. Crit. Care Med. 159 (2), 487–494 (PubMed PMID: 9927362).

Trajkov, D., Mirkovska-Stojkovikj, J., Arsov, T., Petlichkovski, A., Strezova, A., Efinska-Mladenovska, O., Sandevska, E., Gogusev, J., Spiroski, M., 2008. Association of cytokine gene polymorphisms with bronchial asthma in Macedonians. Iran J. Allergy Asthma Immunol. 7 (3), 143–156.

Virchow Jr., J.C., Kroegel, C., Walker, C., Matthys, H., 1996. Inflammatory determinants of asthma severity: mediator and cellular changes in bronchoalveolar lavage fluid of patients with severe asthma. J. Allergy Clin. Immunol. 98, S27–S33.

Witte, J.S., Palmer, L.J., O'Connor, R.D., Hopkins, P.J., Hall, J.M., 2002. Relation between tumour necrosis factor polymorphism TNFalpha-308 and risk of asthma. Eur. J. Hum. Genet. 10 (1), 82–85.

Wjst, M., Fischer, G., Immervoll, T., Jung, M., Saar, K., Rueschendorf, F., Reis, A., Ulbrecht, M., Gomolka, M., Weiss, E.H., Jaeger, L., Nickel, R., Richter, K., Kjellman, N.I., Griese, M., Von Berg, A., Gappa, M., Riedel, F., Boehle, M., Van Koningsbruggen, S., Schoberth, P., Szczepanski, R., Dorsch, W., Silbermann, M., Wichmann, H.E., et al., 1999. A genome-wide search for linkage to asthma. German asthma genetics group. Genomics 58 (1), 1–8.

Yalçın, S., Kayaaltı, Z., Söylemezoğlu, T., 2011. Role of interleukin-6 -174 G/C promoter polymorphism in trace metal levels of autopsy kidney and liver tissues. Int. J. Hyg. Environ. Health 214 (3), 219–224. http://dx.doi.org/10.1016/j.ijheh.2011.01.005 (Epub 2011 Feb 17. PubMed PMID: 21333594).

Ying, S., Robinson, D.S., Varney, V., Meng, Q., Tsicopoulos, A., Moqbel, R., Durham, S.R., Kay, A.B., 1991. Hamid Q.TNF alpha mRNA expression in allergic inflammation. Clin. Exp. Allergy 21 (6), 745–750.

Yokoyama, A., Kohno, N., Fujino, S., Hamada, H., Inoue, Y., Fujioka, S., Ishida, S., Hiwada, K., 1995. Circulating interleukin-6 levels in patients with bronchial asthma. Am. J. Respir. Crit. Care Med. 151 (5), 1354–1358 (PubMed PMID: 7735584).

Yu, L., Yang, M.S., Zhao, J., Shi, Y.Y., Zhao, X.Z., Yang, J.D., Liu, Z.J., Gu, N.F., Feng, G.Y., He, L., 2004. An association between polymorphisms of the interleukin-10 gene promoter and schizophrenia in the Chinese population. Schizophr. Res. 71 (1), 179–183.

Zedan, M., Settin, A., Farag, M.K., El-Bayoumi, M., El Regal, M.E., El Baz, R., Osman, E., 2008. Gene polymorphisms of tumor necrosis factor alpha-308 and interleukin-10-1082 among asthmatic Egyptian children. Allergy Asthma Proc. 29 (3), 268–273.

Zeyrek, D., Demir, E., Alpman, A., Ozkinay, F., Gulen, F., Tanac, R., 2008. Association of interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms in Turkish children with atopic asthma. Allergy Asthma Proc. 29 (5), 468–474.

Zhang, J., Chen, H., Hu, L., Fu, J., Zhang, H., Chen, Y., 2002. Correlation between polymorphism of IL-4 and IL-10 gene promoter and childhood asthma and their impact upon cytokine expression. Zhonghua Yi Xue Za Zhi 82 (2), 114–118.

Zhang, Y., Zhang, J., Tian, C., Xiao, Y., He, C., Li, X., Bogati, A., Huang, J., Fan, H., 2011. The -308 G/A polymorphism in TNF-α gene is associated with asthma risk: an update by meta analysis. J. Clin. Immunol. 31 (2), 174–185. http://dx.doi.org/10.1007/s10875-010-9477-3 (Epub 2010 Nov 17. PubMed PMID: 21082225).