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Original Research Article

Association study between-137 G/C IL-18 polymorphism and asthma in a sample of Algerian population

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Abstract

Like other allergic diseases, asthma results from multiple conditions. Its susceptibility and severity are mediated by both environmental and genetic factors. In asthma studies, the important work is realization of the genetic background and identification of genetic factors resulting in asthma development and phenomena. Here, we investigated whether the single nucleotide polymorphism IL-18 (-137G/C) is associated with asthma in Algerian population. IL-18 (-137G/C) SNP was detected by polymerase chain reaction with sequence specific primers analysis (PCR-SSP) in 170 patients with asthma and 170 normal controls. Our results showed a significant difference in the genotype distribution of IL-18 SNP between asthma patients and controls (χ^2 = 10.80, *P* = 0.005). The Allelic frequency of the G allele was significantly higher in the asthma group compared to the controls (χ^2 = 10.468, *P* = 0.001, OR = 1.694, 95% CI (1.230-2.335). The results revealed that there is a possible association of IL-18 (-137G/C) with asthma in the studied population. This polymorphism can be considered as a marker of susceptibility to asthma. More studies in larger populations are needed to confirm our results.

Key words: Algerian population, Asthma, Cytokines, Polymorphism, IL-18 (-137 G/C)

INTRODUCTION

Asthma is a multifactorial respiratory disease caused by a interaction of multiple susceptibility genetic and environmental factors, which is associated with Th2 and Th1 cytokines (Ma et al., 2012).

Whole-genome scan analyses have revealed that SNP in chromosomal regions 11p15, 11q13, 11q12–13 (Daniels et al., 1996) and 11q22 (Koppelman et al., 2002) are possibly linked to asthma and atopy (Liang et al., 2005). Besides, interleukin 18 (Il-18), located on chromosome 11q22, 2-22,3, it regulates the Th1/Th2 balance, and it is considered as a candidate asthma susceptibility gene (Lachheb et al., 2007). IL -18 is a pro-inflammatory cytokine that is produced by a wide range of cells such as monocytes, macrophages and dendritic cells (Sivalingam et al., 2003). IL-18 is a member of the IL-1 family that was originally described as IFN-γ-inducing factor; it is generally considered to be involved in the T-helper type 1 mediated immune response and it inhibits IgE synthesis, usually by acting synergistically with IL-12. IL-18 is essential for the host's defense against bacteria, fungi, protozoa and viruses through the induction of host-derived IFN-γ. On the other hand, IL-18 might also initiate Th2 responses with production of IgE via the stimulation of IL-4 and IL-13 synthesis in mast cells and basophils under certain conditions, as measured by Th2 cytokine production (Kim et al., 2007). Being involved in the pro -inflammatory cytokine network, IL-18 could have an important role in the development of inflammatory

diseases including asthma. The involvement of this cytokine in atopic disorders was supported by clinical and epidemiological observations of increased circulating IL-18 levels in the serum of patients during acute phase of asthma (Giedraitis et al., 2001). It has been also suggested that IL-18 may play a potential role to activate immunologic responses and may reflect disease activity in mild and moderate asthma exacerbation (Mahajan and Mehta, 2006).

IL-18 gene promoter polymorphism genotypes have been associated with variable levels of IL-18 mRNA production and thus IFN-γ production (Stassen et al., 2003). The search for nucleotide variations in the promoter region of the gene, able to affect IL-18 synthesis and IFN-γ production, resulted in the discovery of several new polymorphisms. One of them is (- 137G>C) transversion, which modifies a transcription binding site influencing the quantity of transcribed mRNA (Boniotto et al., 2005). This SNP has been shown to be associated with asthma in several populations (Liang et al., 2005; Arimitsu et al., 2006; Novak et al., 2005), but no previous study has reported the association between this polymorphism and asthma in Algeria. Because of that, the aim of this work was to study the status IL-18 (-137G>C) polymorphism and its potential association with asthma in Algerian population.

SUBJECTS AND METHODS

Subjects

A total of 170 diagnosed asthmatic patients (68 males and 102 females) and a total of 170 control subjects (108 males and 62 females) were included in this study. All subjects were chosen from those visiting CHUO hospital in western Algeria.

The asthmatic subjects are patients who suffer from cough, wheezing and shortness of breath, and they are visiting the emergency unit for nebulization with typical symptoms of asthma. The controls are healthy subjects who don't have any history of asthma and do not suffer from any chronic disease and did not have any allergic disease. Written informed consent was obtained from all participating subjects.

Blood collection

5 milliliters of peripheral blood samples were collected by specialized nurses in the hospital and stored at -20°C until DNA extraction.

Genomic DNA extraction

The genomic DNA from the blood subjects was extracted using a genomic DNA extraction kits (StratageneInc, Canada) as per manufacturer procedure. The quality of the extracted DNA was verified by running on 1.5% agarose gel and detected by standard ethidium bromide staining. The DNA samples were then stored at 4 °C until needed.

Genotyping

The IL-18 (-137G>C) genotyping was performed using polymerase chain reaction with specific primers (PCR-SSP) assay, which uses identical amplification and detection conditions, enabling rapid and cost-efficient analysis of polymorphisms (Bagheri et al., 2006). This technique utilizes sequence specific primers with 3'-end mismatches and identifies the presence of specific allelic variants through the PCR amplifications (Janssen et al., 2004). a common reverse primer, 5'-AGG AGG GCA AAA TGC ACT GG-3', and two sequence-specific forward primers, 5'-CCC CAA CTT TTA CGG AAG AAA AG-3' and 5'-CCC CAA CTT TTA

CGG AAG AAA AAC-3' were used to amplify a 261 bp PCR product. A control forward primer, 5'-CCA ATAGGA CTG ATT ATT CCG CA-3', was used to amplify a 446 bp fragment covering the polymorphic site as an internal positive amplification control (Boraska et al., 2006). All PCR reactions were performed in a total volume of 25 µl, containing 2 µl genomic DNA, 2.5 µl of 10X Taq polymerase buffer, 2U of taq polymerase, 200µM of dNTPs, 0.25 µM of the control forward primer and each sequence specific primer and 0.5 µM of common reverse primer. PCR reactions were performed in thermocycler (Eppendorf, Germany) according to the following cycling conditions:

2 min at 94 °C, followed by five cycles for 20 s at 94 °C, 1 min at 68 °C and 25 cycles of 20 s at 94 °C, 40 s at 62 °C, 40 s at 72 °C and a final elongation at 72 °C for 5 min. All PCR products were separated in 1.5 % agarose gels stained with ethidium bromide. Gels were visualized under UV transillumination.

Statistical analysis

Data were analyzed with SPSS 21.0 software. All genotype frequencies were tested for the Hardy-Weinberg equilibrium the fit to the equilibrium was tested by calculating the χ^2 test. Comparison of allelic frequencies and genotypes among groups, and association of this polymorphism with asthma were examined for statistical significance with Chi square (χ^2) test. The quantification of the relative risk, the odds ratio (OR) and the confidence interval (CI) were calculated at the 95% level. Statistical significance was assumed for *P* values less than 0.05

Table 1. Baseline characteristics of all the subjects.

Parameters	Case (n=170) N(%)	control (n=170) N(%)	P-value
Gender, M/F	68/102 (40/60)	108/62 (64/36)	NS
Age Parental	45.35±14,326	35.62±12,033	
consanguinity	45 (26)	28 (16)	P<0.05
Personnal history	144 (85)	46 (27)	P<0.05
Professional	65 (38)	31 (18)	P<0.0001
Pollution			
Air pollution	107 (63)	25 (15)	P<0.0001
Professional pollution	108 (64)	47 (28)	P<0.0001
Humidity	129 (76)	90 (53)	P<0.0001
Tobacco smoke	41 (24)	78 (46)	P<0.0001
Passive smoke	54 (32)	94 (55)	P<0.0001
Urban / Rural	151/19 (89/11)	146/24 (86/14)	NS
Environment Presence	40 (24)	22 (13)	P<0.01
Animals at home			
Parental history asthma	70 (41)	35 (21)	P<0.0001

Table 2. The genotype and allele frequencies of IL-18 (-137G>C) polymorphism in asthma patients and healthy controls

polymorphism	Controls N=170 (%)	Asthma Patients N= 170 (%)	χ^2	*P* value	OR (95% CI)
Genotype					
GG	59 (35%)	87 (51%)			
GC	86 (50%)	70 (41%)	10.80	0.005	
CC	25 (15%)	13 (8%)			
Allele					
G	204 (60%)	244 (72%)	10.468	0.001	1.694
C	136 (40%)	96 (28%)			(1.230-2.335)

RESULTS

Baseline characteristics

Patients and healthy controls characteristics are shown in Table 1. The mean age of patients was 45.35±1.099 Versus 35.62±0.923 in the control group, and it was statistically different between the two groups (p<0.005). There was no statistical difference between asthma patients and controls in term of gender and the environment (rural or urban) factors. However there was a significant difference with respect to the other characteristics.

Detection of the IL-18 (-137 G>C) polymorphism

Genotype and allele frequencies for IL-18 (-137G>C) polymorphism are summarized in table 2. The genotype frequencies were in agreement with the Hardy-Weinberg equilibrium.

The frequencies of the three genotypes among control subjects were as follows: GG in 59 patients (35%), GC in 86 patients (50%) and CC in 25 patients (15%).

Whereas the frequencies among asthma patients were: GG in 87 subjects (51%), GC in 70 subjects (41%) and CC in 13 subjects (8%).

Genotypes frequencies distribution was significantly different in asthma patients and controls (χ^2 = 10.80, *P* = 0.005). The homozygous genotype GG was significantly higher in asthmatic patients compared to the control group. Carriers of the G allele were significantly more frequent in asthmatic group than in controls (χ^2 = 10.468, *P* = 0.001). We can suggest that the asthma patients carrying the -137 G allele exhibited an increased risk of developing asthma than controls (OR = 1.694, 95% CI

(1.230 -2.335))(table 2).

DISCUSSION

In the current study we investigated the association of one promoter SNP of the IL -18, which is -137G/C with asthma in Algerien population.

IL-18 is a proinflammatory cytokine that have a promoting or inhibitory effect on the pathogenesis of asthma (Okamura et al., 1995; Dinarello, 1999). IL-18 acts synergistically with IL-12 to promote immune responses of the Th1 cell to produce IFN-γ and to inhibit IgE production (Kim et al., 2007). However, in the absence of IL-12, IL-18 can induce IL-4, IL-13, and histamine release by basophils and mast cells, Th2 differentiation by T cells, and IL-4 production by NK cells and NKT cells (Yoshimoto et al., 1999; Yoshimoto et al., 2000). It was also shown that IL-18 causes Th1 cells to produce IL-13, eotaxin, GM-CSF, and RANTES, which led to eosinophilic inflammation and bronchial hyper-reactivity in a mouse (Sugimoto et al., 2004). It has been suggested that IL_18 may play an important role in the pathophysiology of patients with asthma. Higher serum levels of IL-18 have been previously identified in asthmatic subjects in comparison to healthy control subjects (Giedraitis et al., 2001; Wong et al., 2001). IL-18 gene maps to the chromosomal region 11q22.2-q22.3 in a region suggested in many studies to be associated with allergic diseases (Koppelman et al., 2002; Ober and Moffatt, 2000). The promoter region of IL-18 gene contains multiple transcription initiation sites. It was reported that IL-18 gene promoter polymorphisms could influence IL-18 mRNA expression and thus affect its serum levels (Giedraitis et al., 2001; Wong et al., 2001; Wen et al., 2014). Position -137 of IL-18 gene promoter fall within a potential histone H4 gene transcription factor 1 (H4TF-1) binding site, with the C allele possibly demolishing the binding site of H4TF-1, as the resulting sequence is similar to a binding site for unknown found in the granulocyte macrophage colony-stimulating factor (GM-CSF) promoter (Takada et al., 2002). The functional consequence of the resulting -137 allele is a dramatic repression of the IL-18 promoter, as well as activation of immune cells and inflammatory response (Giedraitis et al., 2001; Wen et al., 2014). IL-18 (-137G/C) polymorphism has been reported to contribute to a variety of inflammatory diseases including asthma, however, conflicting results have been observed in various populations. Indeed, Berbian et al studied the association of IL-18 (-137G/C) polymorphism with asthma in Indian population, the authors found a significant association of this polymorphism with asthma in the studied population (Birbian et al., 2013). Additionnaly, in a German study, a population suffering from atopic eczema was genotyped for IL-18 _137G/C promoter 1 polymorphism revealing significant association, with the highest allelic frequency in patients with intrinsic and non-atopic eczema, especially in patients with low serum IgE levels (Novak et al., 2005). In the same population, the SNPs in exon 1 (+133T/G and +127C/T), promoter 1 (-137G/C) and in promoter 2 (_133C/G), showed significant associations with high IgE levels ($p < 0.005$) conferring increased risk towards allergic rhinitis (Kruse et al., 2003). Also in a Japanese population, IL-18- 137G/C was positively associated with asthma and it was inferred that there was genetic tendency to produce higher levels of IL-18 cytokine by monocytes with _137G/C genotype, which may contribute to the development of asthma and allergic phenotypes (Arimitsu et al., 2006). The association between _137G/C polymorphism has also been confirmed with atopic asthma in a Swiss study (Imboden et al., 2006b).

On the contrary, some studies report no association of the -137 G/C polymorphism with asthma, as in a Jordanian (Attab et al., 2008), German [30], and Singaporean Chinese Malays populations (Liang et al., 2005). Further, a meta-analysis conducted in the people republic of china on five studies inducting 1883 cases and 6645 controls reported no significant association between asthma risk and the -137G/C polymorphism (Ma et al., 2012).

In the present study a comparison of the genotype frequencies of the -137 G/C polymorphism between patients with asthma and controls reveals a significant differences ($\chi^2 = 10.80$, $P = 0.005$), moreover allelic frequency of the C allele was significantly higher in asthmatic group ($\chi^2 = 10.468$, $P = 0.001$). our finding are in concordance with observations by Novak et al, which revealed a significant association of this SNP with atopic eczema in a German population ($p<0.001$, OR= 4.2824, 95% CI (1.2413-14.7716) (Novak et al., 2005), similarly Pawlik et al showed a significant association of -137 C allele with the mild and moderate asthma in a Polish population ($p= 0.006$) (Pawlik et al., 2007). Kruse et al investigated the relationship between IL-18 (-137G>C) polymorphism and specific sensitization to common allergens and allergic rhinitis, the authors observed a significant association of this polymorphism and IgE levels and specific sensitization ($p<0.005$) (Kruse et al., 2003).

CONCLUSION

Our results suggest that the C allele might be a risk factor for asthma in this population. However, since our sample size was relatively small, association or linkage study in larger sample size is needed to confirm the obtained results. Also, future studies should include the relatives of asthma patients to be certain the association between this SNP and the disease. Moreover, it may be interesting to investigate the implication of other polymorphisms in IL-18 gene that are in linkage disequilibrium with the IL-

18 (-137G/C) polymorphism and that might be responsible for the noted association.

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Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regard- ing the publication of this article

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