Lack of association between adult asthma and the tumour necrosis factor α-308 polymorphism gene

R. Louis*, E. Leyder*, M. Malaise#, P. Bartsch*, E. Louis#


ABSTRACT: Tumour necrosis factor (TNF)α is a cytokine endowed with potent inflammatory properties that may contribute to airway inflammation in asthma. It has previously been shown that the single base pair polymorphism-308 (G to A substitution) in the promoter of TNFα gene results in enhanced cytokine secretion. Whether this polymorphism is associated with the presence of phenotypic expression of asthma is questioned.

In this study the relative frequency of TNF1 and TNF2 alleles in a population of adult healthy subjects (n=98) and adult Caucasian asthmatics (n=95) was compared taking into account their disease severity, atopic status and their smoking habit.

For the whole group of asthma the genotype frequency for 1/1, 1/2, 2/2 were 67%, 33% and 0%, respectively, and not significantly different from those found in the control group that reached 70%, 28% and 2% respectively (p>0.05). The allele frequencies in asthma were 86% and 14% for TNF1 and TNF2 respectively while the corresponding figures were 85% and 15% in the control group (p>0.05). Furthermore, subdividing asthmatics into severe forced expiratory volume in one second <60% pred), atopic or smoking patients did not show any significant association with this TNFα polymorphism.

To conclude the polymorphism -308 in the promoter of the TNFα gene does not confer a susceptibility to develop asthma nor to grade its severity.


Even if the role of environment is critical in the clinical expression of asthma, epidemiological studies have firmly established a genetic component in this disease. However, the genes involved in asthma are still unknown and are likely to be numerous. One way to unravel the potentially important genes in polygenic disorders such as asthma is to perform association studies with candidate genes [1–3].

Over the past 15 yrs the airways inflammatory component of asthma has been clearly demonstrated and accumulating evidence suggests that the immune disorder leading to airways inflammation is partly controlled by the genetic background of the patient [4]. Tumour necrosis factor (TNF)α is a potent proinflammatory cytokine the altered secretion of which could contribute to the inflammatory process in asthmatic airways [5]. A functional polymorphism for TNFα has been recently described. It is characterized by a single base pair (SBP) polymorphism at position -308 in the promoter of TNFα [6]. The mutation leading to the allele 2 has been shown to be associated with an enhanced transcription of TNFα [7] as well as increased release of TNFα from blood mononuclear cells [8] or whole blood [9] in response to endotoxin.

The first two studies looking at this polymorphism in a population of atopic asthma yielded contradictory results [10, 11]. One of these used a validated and standard questionnaire to define asthma in nuclear families [10] while the other one, which exclusively targeted childhood asthma, included the notion of histamine bronchial hyperresponsiveness in the asthma definition [11].

In this study the frequency of TNFα-308 polymorphism in a population of adult asthmatics seen in an outpatient clinic at the University hospital has been assessed. In order to increase the specificity of the diagnosis, asthma was not only defined by the clinical history but also by the demonstration of a significant reversibility of airway obstruction after either inhaled bronchodilator or a short course of oral corticoids, or a significant methacholine bronchial hyperresponsiveness in case of normal baseline bronchial calibre. In order to assess any potential association between TNFα polymorphism and different asthma phenotypes, the patients were further characterized with respect to their atopic status, their smoking history and their disease severity as judged by the extent of lung function impairment.

Materials and methods

Subjects

One hundred unrelated Caucasian asthmatics, whose characteristics are given in table 1, were consecutively recruited for blood sampling by the same physician from the outpatient clinic of Liège University Hospital between
April 1996 and April 1997. Asthma was diagnosed as a clinical history of recurrent episodes of breathlessness, cough and/or wheeze associated either with methacholine bronchial hyperresponsiveness, when baseline FEV₁ was ≥70% pred and/or with a reversibility of bronchial obstruction when baseline FEV₁ was <80% predicted. Methacholine bronchial challenge was carried out by using a jet nebuliser the characteristics of which have been described previously [12]. The subjects inhaled by tidal breathing for 2 min four-fold increasing concentrations of methacholine starting with 0.06 mg mL⁻¹ and going up to 16 mg mL⁻¹ when necessary. FEV₁ was measured 60 s after each inhaled concentration and the challenge stopped when FEV₁ fell by >20% from baseline. The provocative concentration of methacholine causing a fall in FEV₁ of 20% from baseline (PC₂₀ M) was calculated (range). M: male; F: female; FEV₁: forced expiratory volume in one second; ND: not detected.

### Table 1. – Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control population</th>
<th>Asthmatics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects n</td>
<td>98</td>
<td>95</td>
</tr>
<tr>
<td>Age yrs</td>
<td>36 (19–58)</td>
<td>41 (17–74)</td>
</tr>
<tr>
<td>Sex ratio M/F</td>
<td>45/53</td>
<td>38/47</td>
</tr>
<tr>
<td>FEV₁ % pred</td>
<td>103 (68–132)</td>
<td>81 (25–127)</td>
</tr>
<tr>
<td>FEV₁ &lt;60% pred</td>
<td>0</td>
<td>18 (19)</td>
</tr>
<tr>
<td>Smoking history</td>
<td>24/98 (24)</td>
<td>46/95 (49)</td>
</tr>
<tr>
<td>Smokers &gt;5 pack yrs</td>
<td>2/98 (20)</td>
<td>39/95 (41)</td>
</tr>
<tr>
<td>Atopy</td>
<td>11/98 (11)</td>
<td>68/95 (72)</td>
</tr>
<tr>
<td>Total serum IgE kU L⁻¹</td>
<td>ND</td>
<td>63 (3.5–6280)</td>
</tr>
</tbody>
</table>

Data expressed as a mean (range) or as frequency. (%) total serum immunoglobulin (Ig)E is expressed as geometric mean (range).

As described by Verjans et al. [14], a polymerase chain reaction (PCR) involving primers specific for each allele of the G to A polymorphism at residue -308 was used. Four primers were used: the 3' primer (Cl, position: -144/-164: 5'-TCTCGGTTTCTTCCATCG-3') was used in combination with either the 5' primer C2 (position -328/-308G: 5'-ATAGGTGGTGAGGGCATG-3'), complementary to the TNF1 allele (TNF1), or the 5' primer C3 (position-328/-308A: 5'-ATAGGTTTTGAGGGCATG-3') complementary to the TNF2 allele (TNF2). For each DNA sample, two parallel reactions were performed. The primer pair C1/C2 were used to produce specific amplification of TNF1; C1/C3 were used to amplify the TNF2 allele. As an internal control, primer D (position -675 to -655: 5'GAGTCCTCGGGTCTCGAATGA-3') was added to each reaction. Amplification was carried out using the cycling conditions previously described [13].

### Tumour necrosis factor-308 single base pair substitution

Genomic deoxyribonucleic acid (DNA) was extracted from 10 mL of venous blood, using a modified salting out technique [13] and resuspended in sterile distilled water at a final concentration of 0.1–1.0 μg mL⁻¹, before use.

### Statistics

Genotype and allele frequencies between groups were compared using a 2X2 contingency table and chi-squared statistics. Corrections for small numbers were made where necessary using Fisher’s exact test. The genotype distributions of the TNFα-308 polymorphisms for both the control and the asthmatic groups were found to be in Hardy-Weinberg equilibrium.

### Results

Ninety-eight control subjects and 95 asthmatics were successfully genotyped (fig. 1). For the whole group of asthmatics the frequency of genotypes 1/1, 1/2 and 2/2 was 67%, 33% and 0%, respectively, while the corresponding figures in the control group were not significantly

Deoxyribonucleic acid extraction

Genomic deoxyribonucleic acid (DNA) was extracted from 10 mL of venous blood, using a modified salting out technique [13] and resuspended in sterile distilled water at a final concentration of 0.1–1.0 μg mL⁻¹, before use.
Data presented as frequency (%).

Among the asthmatics 28 out of 95 subjects (29%) reported asthma during the childhood (<15 yrs) but there was no difference in the frequencies of TNF1 and TNF2 alleles between those who had childhood onset compared to those who had adult onset of the disease (p>0.05). Among the latter 20 out of 67 (30%) had their first symptoms of asthma after the age of 50, but their TNFα-308 genotype was not different from that found in those in whom the disease started in the middle age (table 4).

Eighteen asthmatics (19%) had a more severe disease as judged by FEV1 values measured twice <60% pred within the last 5 yrs despite regular use of inhaled corticosteroids. Allele frequencies for TNF1 and TNF2 were 81 and 19%, respectively, and not different from those found in mild to moderate asthmatics (84% and 16%, respectively) or control subjects (table 5).

Based on skin prick tests, 68 asthmatics were found to be atopic, whereas 27 were considered as intrinsic. Intrinsic asthmatics were slightly older (mean±SEM age: 49±2.5 yrs versus 38±1.5 yrs, p<0.01) and had lower serum IgE (geometric mean (range): 27 kU.L⁻¹ (<3.5–1660) versus 93 kU.L⁻¹ (<3.5–6230), p<0.05) than their atopic counterparts. The two groups did not differ significantly from each other by their sex ratio, FEV1 values, or their tobacco consumption, but the proportion of patients reporting a childhood onset tended to be lower in intrinsic asthmatics than in atopic asthmatics (4 out of 27; (15%) versus 24 out of 68 (35%) p=0.08). In atopic asthma the TNF1 and TNF2 alleles frequencies reached 87 and 13%, respectively, while corresponding figures in intrinsic asthma were 82 and 18% (p<0.05) (table 6). In asthmatics whose total serum IgE was beyond the normal range (>114 kU.L⁻¹, n=34) the TNF2 frequency was 20% and not significantly different from the 15% found in asthmatics whose total serum IgE was within the normal range (n=41).

Finally, 49% (46/95) of asthmatics were current or exsmokers but those asthmatics with a smoking history displayed exactly the same frequencies for TNF1 and TNF2 alleles (84% and 16%, respectively) as those who had never smoked. In addition smoking asthmatics did not differ significantly from smoking control subjects (table 7).

**Discussion**

In this study no significant association between adult asthma and a polymorphism located in TNFα gene promoter at position -308 was found and the authors were able to influence TNFα secretion in various experimental conditions [7–9]. In addition, the lack of association persisted irrespective of the asthma phenotypes.

The present study, which is the first to be conducted on European Caucasians, yields results which are at variance with those obtained in other populations. This may be due to genetic differences between populations, or to the influence of other confounding factors such as smoking status, age of onset of disease, etc.

**Table 3.** Allele frequency for tumour necrosis factor (TNF)α-308 polymorphism (alleles TNF1 and TNF2) in asthma versus control subjects

<table>
<thead>
<tr>
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<th>TNF1</th>
<th>TNF2</th>
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<tbody>
<tr>
<td>Control population (n=98)</td>
<td>167/196 (85)</td>
<td>31/196 (15)</td>
</tr>
<tr>
<td>Asthma (n=95)</td>
<td>159/190 (84)</td>
<td>31/190 (16)</td>
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</table>

Data presented as frequency (%).

**Table 4.** Allele frequency for tumour necrosis factor (TNF)α-308 polymorphism (alleles TNF1 and TNF2) in asthma according to the onset of the disease

<table>
<thead>
<tr>
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<th>TNF1</th>
<th>TNF2</th>
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<tbody>
<tr>
<td>Control population (n=98)</td>
<td>167/196 (85)</td>
<td>31/196 (15)</td>
</tr>
<tr>
<td>Asthma onset &lt;15 yrs (n=28)</td>
<td>48/56 (86)</td>
<td>8/56 (17)</td>
</tr>
<tr>
<td>Asthma onset 15–50 yrs (n=47)</td>
<td>77/94 (82)</td>
<td>17/94 (18)</td>
</tr>
<tr>
<td>Asthma onset &gt;50 yrs (n=20)</td>
<td>34/40 (85)</td>
<td>6/40 (15)</td>
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</table>

Data presented as frequency (%).
with the previous studies assessing the association between the TNF\(\text{\textalpha}\)-308 polymorphism and the prevalence of childhood asthma in Australia [10, 11]. Although discrepancies between the prescribed results and those of Moffat et al. [10] and Albuquerque et al. [11] might be explained by a different asthma phenotype (adult versus childhood asthma) these two studies yielded conflicting results. Indeed Moffat et al. [10] reported an increased prevalence of TNF\(\text{\textalpha}\) allele in asthma while Albuquerque et al. [11] found the TNF\(\text{\textalpha}\) allele to be more frequently associated with the disease. In the former study which included 92 asthmatics, the frequency of TNF\(\text{\textalpha}\) reached 29\% compared to 16\% in the present study. It is of importance to note that the TNF\(\text{\textalpha}\) frequency in the present control group (15\%) was very close to that found by Moffat et al. [10] in their large cohort (n=312) of nonasthmatic subjects (17\%). This suggests that the lack of association between adult asthma and TNF\(\text{\textalpha}\)-308 polymorphism found is not due to a bias in the selection of control population. It could be argued that the asthmatics identified from a questionnaire sent to a general population as in the study could be argued that the asthmatics identified from a bias in the selection of control population. It

### Table 5 – Allele frequency for tumour necrosis factor (TNF)\(\text{\textalpha}\)-308 polymorphism (alleles TNF1 and TNF2) according to asthma severity

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<th>TNF1</th>
<th>TNF2</th>
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</thead>
<tbody>
<tr>
<td>Control population (n=98)</td>
<td>167/196 (85)</td>
<td>31/196 (15)</td>
</tr>
<tr>
<td>Asthma with FEV(_1) ≥60% (n=77)</td>
<td>130/154 (84)</td>
<td>24/154 (15)</td>
</tr>
<tr>
<td>Asthma with FEV(_1) &lt;60% (n=18)</td>
<td>29/36 (81)</td>
<td>7/36 (19)</td>
</tr>
</tbody>
</table>

Data presented as frequency (%). FEV\(_1\): forced expiratory volume in one second.

Asthmatics were considered to be severe if they displayed FEV\(_1\) values <60\% twice in the last 5 yrs, despite regular treatment with inhaled corticoids. The possibility of a particular association between TNF\(\text{\textalpha}\) and severe asthma was examined for at least two reasons. The first is that such an association has been recently described for a subgroup of severe inflammatory bowel disease, the pathogenesis of which may somehow resemble that of asthma [16]. Secondly the inflammatory picture within the Airways of severe asthmatics, involving both eosinophil and neutrophil (as opposed to the eosinophils alone in the mild to moderate forms of the disease) [17–19] fits with a role for TNF\(\text{\textalpha}\) that favours the tissular recruitment of both type of granulocytes [20] partly through endothelial expression of ICAM-1 [21]. However the present results fail to show any preferential association between TNF\(\text{\textalpha}\) and severe asthma, even after excluding the smoking asthmatics. The lack of difference between mild to moderate and severe asthma found here in agreement with a recent study conducted in a large population from the USA, although in that study the authors reported a slight increase in the prevalence of genotypes 1-2 and 2-2 in 150 asthmatics, when compared to a random population (40 versus 30\%) [22]. Interestingly in the random population of that study, the prevalence of genotypes, including at least one TNF\(\text{\textalpha}\) allele (genotype 1-1 and 1-2), is similar to the figure found in the present control population (30\%), which adds consistency in the interpretation of the data.

The present data do not demonstrate this TNF\(\text{\textalpha}\) polymorphism to be associated with atopy as defined by skin prick test to common aeroallergens nor with abnormally high total serum IgE levels, a phenotype which was previously shown to be linked to a mutation in the gene of Fcr\(_\text{RI}\) [23]. Although the definition of atopy was restricted to skin prick test interpretation and not based on suggestive symptoms in the present study, it is believed that not many real atopic patients who could have been skin prick test negative for the two following reasons were missed. Firstly, because the mean age in our intrinsic asthmatics was 49 yrs, an age at which skin prick test are still regularly positive in atopic patients; secondly, because the intrinsic asthmatics had, on average, lower total serum IgE levels than atopic patients, clearly highlighting the real immunonological difference between the two groups.

Interestingly, almost one half of the patients diagnosed as having asthma on the basis of clinical symptoms and

### Table 6 – Allele frequency for tumour necrosis factor (TNF)\(\text{\textalpha}\)-308 polymorphism (alleles TNF1 and TNF2) in asthma according to the atopic status

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<th>TNF1</th>
<th>TNF2</th>
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</thead>
<tbody>
<tr>
<td>Control population (n=98)</td>
<td>167/196 (85)</td>
<td>31/196 (15)</td>
</tr>
<tr>
<td>Atopic asthma (n=68)</td>
<td>112/136 (82)</td>
<td>24/136 (18)</td>
</tr>
<tr>
<td>Intrinsic asthma (n=27)</td>
<td>47/54 (87)</td>
<td>7/54 (13)</td>
</tr>
</tbody>
</table>

Data presented as frequency (%).
either bronchial hyperresponsiveness to methacholine or a significant reversibility of airway obstruction, were current or ex-smokers. As no difference in the TNF-α-308 allelic frequencies have been found between smoking and non-smoking asthmatics, the lack of association between asthma and this TNF-308 gene polymorphism in this study does not result from the fact that smokers were included in the population of asthmatics. Furthermore, the lack of difference between control smokers and asthmatic smokers suggests that TNF-308 polymorphism does not make smokers more prone to develop asthma. This observation can be compared with a very recent study by Higham et al. [24] showing that this polymorphism was not a risk factor for the susceptibility to chronic obstructive pulmonary disease in European Caucasian smokers.

In conclusion the data fail to show any significant association between asthma and the polymorphism at position -308 in the promoter of the TNFα gene, suggesting that this polymorphism is unlikely to play an important role in asthma pathophysiology. This conclusion holds for different phenotypes of asthma including the severe form of the disease.

References