

Association of tumor necrosis factor- α –308G > A polymorphism with IgE-mediated allergy to betalactams in an Italian population

R-M Guéant-Rodriguez^{1,4},
J-L Guéant^{1,4}, M Viola²,
D Tramoy¹, F Gaeta² and
A Romano^{2,3}

¹Inserm U-724, Faculté de Médecine, University of Nancy-Henri Poincaré and University Hospital Center (CHU) of Nancy, Nancy, France;

²Department of Internal Medicine and Geriatrics, UCSC-Allergy Unit, Complesso Integrato Columbus, Rome, Italy and ³IRCCS Oasi Maria SS, Troina, Italy

Correspondence:

Dr J-L Guéant, Inserm U-724, University of Nancy-Henri Poincaré, BP 184, F- 54505, Nancy-Vandoeuvre, France.
E-mail: jl.gueant@chu-nancy.fr

Tumor necrosis factor- α (TNF- α) is released from mast cells via an immunoglobulin E (IgE)-dependent mechanism. The variant G > A at –308 of *TNFA* is part of an extended haplotype HLA-A1-B8-DR3-DQ2 and influences the gene expression. We evaluated this variant in relation to IgE-mediated reactions to betalactams, in 427 subjects, including 167 cases and 260 age- and gender-paired controls. *TNFA* GG genotype was a significant independent predictor of the primary risk of betalactam allergy, concurrently with total IgE level, with an age- and sex-adjusted odds ratio estimated at 2.45 (95% confidence interval: 1.18–5.08, $P=0.0163$). Cases with –308AA genotype had a higher serum level of specific IgE than those with –308GA/GG genotype, with median levels (relative units) of 4.6 (inter-quartiles: 3.9–10.6) and 2.2 (1.4–4.3), respectively ($P=0.0046$). In conclusion, our results suggest an ambivalent influence of a genetic determinant of pro-inflammatory pathways on IgE-mediated hypersensitivity to betalactams.

The Pharmacogenomics Journal advance online publication, 1 May 2007; doi:10.1038/sj.tpj.6500456

Keywords: IgE; betalactams; allergy; genetic polymorphism; TNFA

Introduction

Penicillins and cephalosporins are the two major classes of the betalactam family and also the antibiotics most widely used for treating common infections.¹ Immediate reactions to betalactams (that is, occurring within 1 h after drug administration), particularly anaphylactic ones, are the most common adverse reactions to antibiotics mediated by a specific immunologic mechanism.^{2,3} The drug (or a drug metabolite) reacts with immunoglobulin E (IgE) bound to the surface of mast cells and leads to the activation, degranulation and release of vasoactive mediators from mast cells and basophiles. Reactions may be induced by all betalactams currently available, ranging from benzylpenicillin to more recent compounds.

The cause of IgE-mediated reactions to betalactams is unclear and may involve several biological phenomena related to antigen presentation, class switching to IgE by B cells and the release of mediators from mast cells and basophiles.⁴ IgE-dependent mast cell activation plays an important role in the development of allergy. The cross-linking of allergen-specific IgE bound to its high-affinity receptor, Fc ϵ RI, results in a series of molecular events leading to nuclear factor (NF)- κ B activation^{5,6} and subsequent cytokine production. Many mast

⁴These authors have contributed equally to this work.

cell-derived cytokines, including tumor necrosis factor- α (TNF- α), are regulated by the transcription factor NF- κ B.⁷

TNF- α is a versatile cytokine that could be potentially involved in different steps of the pathomechanisms of IgE-mediated reactions to betalactams. TNF- α is released from both mast cells and macrophages via IgE-dependent mechanisms. Its production was first described in macrophages and monocytes⁸ and the mast cell granule itself contains preformed TNF- α .^{9,10} This cytokine plays a key role in the development and adaptive responses of the immune system, but its influence on IgE production is unknown.¹¹ It may also influence the severity of the reaction *via* its effects on epithelial cell barrier permeability, recruitment and activation of macrophages and mast cells.¹¹

Single-nucleotide polymorphisms (SNPs) in regulatory regions of cytokine genes have been associated with susceptibility to a number of complex disorders. An SNP located at nucleotide -308 with respect to the *TNFA* transcriptional start site corresponds to a substitution of adenine for guanine. This variant of the promoter region seems to be the one having the strongest phenotypic influence among the different polymorphisms of *TNFA* promoter and it had been found associated with susceptibility to cerebral malaria, with leishmaniasis, meningococcal infections and asthma.^{12–17} Recently *TNFA* -308A allele has also been associated with carbamazepine allergy.¹⁸

On the basis of the increasing interest for evaluating the role of TNF- α in allergy, we investigated the *TNFA* -308G>A polymorphism in relation to IgE-mediated reactions to betalactams in a case-control study. In particular, we evaluated its association with the primary risk, the severity of the reaction, the level of total and specific serum IgE and the skin test positivity.

Results

Our cases of immediate allergic reactions to betalactams were compared to a control population without reported allergic disease. Both groups had close age range and gender distribution. The median age of patients and controls at inclusion were not significantly different (Table 1). The sex ratio was similar in the allergic group and the control group (Table 1). The patients had a significantly higher concentration of total serum IgE than the controls (Table 1) and none of the controls had either a total IgE serum level higher than 150 UI/ml or the presence of specific IgE.

The genotype distribution of *TNFA* -308G>A polymorphism was in Hardy-Weinberg equilibrium in controls. The frequencies of the predominant -308G allele of *TNFA* and the *TNFA* GG genotype were significantly higher in patients than in controls (Table 2). The *TNFA* GG genotype was a

Table 1 Clinical and biological characteristics of patients with IgE-mediated allergy to betalactams

Characteristic	Patients (n = 169)	Controls (n = 265)	P-value ^a
Number of cases (% , 95% confidence interval)			
Age ^b	40 (24–55)	45 (29–58)	0.1197
Female gender	115 (68.0, 60.7–74.6)	166 (63.8, 57.8–69.4)	0.3711
Time interval between the reaction and the evaluation (months) ^b	6 (2–18)	0	
Personal history of allergy	49 (28.9, 22.7–46.3)	0	
Family history of allergy	56 (33.1, 26.5–40.6)	0	
Culprit drug			
Penicillin	95 (56.2, 48.7–63.5)	0	
Cephalosporin	74 (43.8, 36.5–51.3)	0	
Clinical presentation			
Urticaria	27 (16.0, 11.2–22.3)	0	
Angioedema or urticaria	22 (13.0, 8.8–18.9)	0	
Anaphylactic shock	120 (71.0, 63.7–77.3)	0	
Skin tests, positive to			
Culprit drug	116 (68.6, 61.3–75.2)	0	
Penicilloyl polylysine	40 (23.7, 17.9–30.6)	0	
Minor determinant mixture	42 (24.9, 18.8–31.7)	0	
Benzylpenicillin	41 (24.3, 18.4–31.3)	0	
Total IgE (UI/ml) ^b	114 (48–269)	20 (7–42)	<0.0001
Positive specific IgE	110 (65.1, 57.6–71.9)	0	

Abbreviation: IgE, immunoglobulin E.

^aCategorical variables were compared using a χ^2 -test and continuous variables using a Mann-Whitney *U*-test.

^bExpressed in median and quartiles.

significant independent predictor of the primary risk of betalactam allergy, concurrently with total IgE level, with respective age- and sex-adjusted odds ratios estimated at 2.45 (95% confidence interval (CI): 1.18–5.08, $P=0.0163$) and 1.02 (95% CI: 1.02–1.03, $P<0.0001$).

Table 3 describes the frequencies of categorical clinical and biological characteristics, according to the allele distribution in patients. No difference of age was reported between the cases carrying the allele G or the allele A (44, 27–57 and 46, 28.5–57 years, respectively, $P=0.6242$). We did not observe any association of *TNFA* -308G>A polymorphism with either the serum level of total IgE, skin test

positivity to culprit betalactams or severity of the reaction, while we found a significant association with the level of anti-betalactam-specific IgE. Indeed, cases with -308AA genotype had a higher serum level of anti-betalactam-specific IgE than those with -308GA/GG genotypes, with median levels (relative units) of 4.6 (inter-quartiles: 3.9–10.6) and 2.2 (1.4–4.3), respectively ($P=0.0046$) (Figure 1).

Table 2 Number and frequency of alleles and genotypes of *TNFA* -308G>A polymorphism in 169 patients with IgE-mediated allergy to betalactams and 260 controls

TNFA -308	Number of cases, frequencies (95% CI)		P-value ^a
	Patients	Controls	
GG	150, 0.89 (0.83–0.93)	206, 0.79 (0.74–0.83)	0.0278
GA	15, 0.09 (0.06–0.14)	47, 0.18 (0.14–0.23)	
AA	4, 0.02 (0.01–0.06)	7, 0.03 (0.01–0.06)	
Allele G	315, 0.93 (0.90–0.95)	459, 0.88 (0.85–0.91)	0.0177
Allele A	23, 0.07 (0.05–0.10)	61, 0.12 (0.09–0.15)	

Abbreviation: CI, confidence interval.

^aCategorical variables were compared using a χ^2 -test.

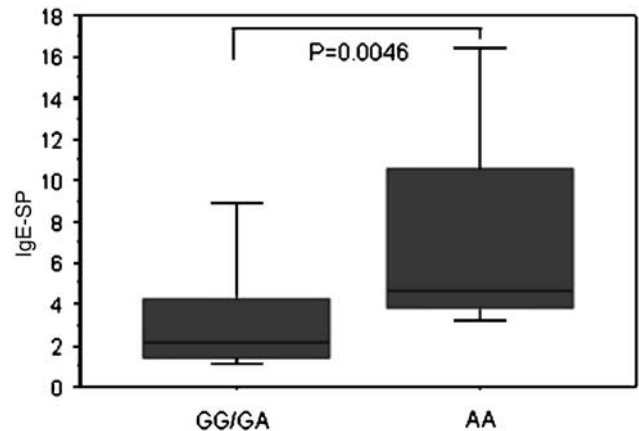


Figure 1 Serum level of specific IgE against culprit cephalosporins and penicillins (box-plots of relative units, with median and interquartiles), according to genotypes of *TNFA* -308G>A polymorphism, in 169 cases with β -lactam IgE-mediated allergy.

Table 3 Comparison of the frequencies of clinical and biological characteristics in the patients carrying either the alleles A or G of the *TNFA* -308A>G polymorphism, in 169 patients with IgE-mediated allergy to betalactams

Characteristic	Frequency (95% CI) of characteristic according to TNFA -308G>A		P-value	Odds ratio ^a (95% CI)
	Patients with allele G	Patients with allele A		
Female sex	0.66 (0.59–0.74)	0.79 (0.67–0.91)	0.4716	1.19 (0.73–1.94)
Personal history of allergy	0.29 (0.24–0.34)	0.32 (0.15–0.55)	0.5262	1.33 (0.54–3.25)
Familial history of allergy	0.34 (0.29–0.39)	0.26 (0.12–0.49)	0.4569	0.69 (0.27–1.82)
<i>Culprit drug</i>				
Penicillin	0.56 (0.50–0.61)	0.61 (0.41–0.78)	0.6410	0.76 (0.31–1.86)
Cephalosporin	0.44 (0.39–0.50)	0.39 (0.22–0.59)	0.6410	0.76 (0.31–1.86)
<i>Clinical presentation of immediate reaction</i>				
Urticaria	0.17 (0.13–0.21)	0.09 (0.02–0.27)	0.5458	0.47 (0.11–2.13)
Angioedema or urticaria	0.13 (0.10–0.17)	0.17 (0.07–0.37)	0.7938	1.29 (0.41–4.06)
Anaphylactic shock	0.70 (0.66–0.75)	0.74 (0.54–0.88)	0.6975	1.29 (0.49–3.40)
<i>Skin tests, positive to</i>				
Culprit drug	0.79 (0.73–0.83)	0.83 (0.63–0.93)	0.6508	1.30 (0.43–3.99)
Penicilloyl polylysine	0.24 (0.20–0.29)	0.17 (0.07–0.37)	0.3465	0.57 (0.16–2.08)
Minor determinant mixture	0.26 (0.22–0.31)	0.09 (0.02–0.27)	0.0633	0.27 (0.06–1.16)
Benzylpenicillin	0.24 (0.20–0.29)	0.26 (0.13–0.47)	0.8323	0.87 (0.33–2.31)
Total IgE >150 UI	0.83 (0.80–0.86)	0.86 (0.77–0.92)	0.5438	1.22 (0.64–2.29)
Positive specific IgE	0.64 (0.58–0.67)	0.87 (0.68–0.96)	0.0227	3.83 (1.12–13.18)

Abbreviations: CI, confidence interval; IgE, immunoglobulin E.

^aAge adjusted.

Table 4 Analysis by stepwise logistic regression^a of the determinants for the risk for the presence of specific IgE in serum from 169 patients with IgE-mediated allergy against betalactams

Variable	Initial ^a			Residual ^a		
	OR	95% CI	P-value	OR	95% CI	P-value
Age	1.02	1.00–1.04	0.0201	1.02	1.00–1.04	0.0260
Female sex	0.61	0.26–1.45	0.2644	—	—	—
Personal history of allergy	1.15	0.46–2.50	0.7554	—	—	—
Familial history of allergy	0.96	0.40–2.29	0.9275	—	—	—
Cephalosporin allergy	1.21	0.52–2.80	0.6548	—	—	—
Anaphylactic shock	1.33	0.56–3.19	0.5197	—	—	—
Time interval	0.99	0.96–1.00	0.2972	—	—	—
Positive skin test	0.52	0.13–2.10	0.3611	—	—	—
Total IgE	1.01	1.00–1.01	<0.0001	1.00	1.00–1.01	<0.0001
TNFA -308A allele	4.74	1.21–18.5	0.0251	4.29	1.11–16.5	0.0343

Abbreviations: CI, confidence interval; IgE, immunoglobulin E; OR, odds ratio.

^aStepwise multivariate logistic regression was performed using a model that included the clinical and biological characteristics as initial variables and retained those with a *P*-value lower than 0.15 in the residual step.

A weak association, at the limit of statistical significance, was also found between *TNFA* -308G>A and the skin tests positive with minor determinants. Twenty-six and 8.7% of the patients who were carrying the allele *G* or *A* had a positive skin test to minor determinants, respectively (*P*=0.0633). In multivariate logistic regression, the age- and sex-adjusted odds ratios for the presence of specific IgE and positive skin testing of minor determinant mixture in allele *A* carriers were estimated, respectively, at 4.25 (95% CI: 1.22–14.80, *P*=0.0232) and 0.25 (95% CI: 0.06–1.10, *P*=0.0671). In multiple regression analysis, *TNFA* -308A allele was a significant predictor of the level of specific IgE in serum (Table 4).

Discussion

We focused our interest on *TNFA*-308G>A polymorphism because of its known involvement in differential transcriptional activator, elevated plasma TNF- α levels and higher amounts of TNF- α on cell stimulation *in vivo* and *ex vivo*.^{19,20} In addition, this polymorphism is in linkage disequilibrium with three other variants of the promoter region of *TNFA* gene, -1031T>C, -863C>A, -857C>T, while a fourth variant, -238G>A, seems to have no phenotypic influence.¹⁶ Despite the relative frequency of immediate allergic reactions to betalactams, one limit in evaluating their correlation with genetic risk factors is the difficulty of recruiting large and well-documented series. The present series had a size compatible with the objective of our study, considering the allele frequency of the *TNFA* -308G>A polymorphism reported in Caucasian populations from Western Europe and Australia.^{15,21,22} The allele frequency of *TNFA* -308G>A polymorphism observed in our control population was close to that previously reported in a large cohort of subjects from two Italian areas.²¹ We observed a higher concentration of total serum IgE in patients than in

controls, suggesting the involvement of a mechanism related to IgE class switching. Our results showed that *TNFA* GG carriers had a twofold increased risk of β -lactam allergy, compared to *TNFA* GA and AA carriers, and that this association was not related to an influence of this polymorphism on the production of total IgE. In agreement with our results, some authors have reported previously an association between *TNFA* GG carriers and asthma but no association with IgE level.^{16,22} Other studies have shown an association between the carriage of *TNFA* -308A allele and infectious diseases, asthma,^{12–17,23–25} contact dermatitis²⁶ and hypersensitivity against carbamazepine,¹⁸ while two groups found no significant association with toluene diisocyanate-induced asthma and atopic asthma, respectively.^{27,28}

The association between *TNFA* -308GG genotype and β -lactam allergy could be due to a mechanism related to antigen presentation.²⁹ Indeed, the TNF receptors modulate HLA antigen expression¹¹ and the *TNFA* gene lies in the class III region of the major histocompatibility complex (MHC), 250 kilobases centromeric of the HLA-B locus and 850 kilobases telomeric of HLA-DR. The variant at -308 of *TNFA* is part of an extended MHC haplotype HLA-A1-B8-DR3-DQ2.³⁰ Our result therefore strengthens the hypothesis that the presentation of penicillin-hapten determinants to lymphocyte involves the MHC in immediate type allergy to β -lactams. Indeed, in a former work, we found a higher prevalence of HLA A2 and DRw52 and a lower frequency of DR4 in patients with hypersensitivity to aminopenicillins.³¹

The association between *TNFA* -308GG genotype and β -lactam allergy could be also due to a molecular mechanism related to the trans-regulation of *TNFA* expression.²⁹ However, the functional importance of this polymorphism seems to vary among cells. No difference between *TNFA* allele *A* and *TNFA* allele *G* promoter activities was found in B-cell line.³² In contrast, *TNFA* allele *A* promoter had a 2- to 3-fold greater transcriptional activity than allele *G* promoter

in stimulated U937 monocytes and Jurkat T cells. Although it is known that TNF- α is released in allergic responses from both mast cells and macrophages via an IgE-dependent mechanism, the relationship of *TNFA* polymorphisms and total serum IgE has not been studied thoroughly. We found a significant association between the carriage of *TNFA* -308A allele and the positivity of specific serum IgE radioimmunoassay (RIA), but not with total serum IgE level nor with positivity of skin testing. In agreement with our data, a lack of association between this variant and total IgE has been reported previously in asthmatic patients.¹⁶ The authors reported an opposite effect of *TNFA* variant on onset and progress of asthma, that is a protective effect on the risk of asthma development and detrimental effect on progress of asthma.¹⁶ Similarly, we observed an ambivalent influence of *TNFA* -308G>A with the risk of betalactam allergy and the production of specific IgE that may be related to CMH antigen presentation and to the trans-regulation of *TNFA*, respectively (Tables 3 and 4). The association between *TNFA* -308A allele and specific serum IgE may be related to the enhancing effect of this variant on TNF- α production by antigen-stimulated mast cells. This enhanced production could have an influence on the Th1/Th2 differentiation and/or proliferation of sensitized B cells. However, experimental data are lacking to document such a hypothesis. Recently, we have shown that *IL13* and *IL4RA* variants are interacting predictors of immediate allergic reactions to betalactams through a mechanism related to IgE switching.³³ The *IL4RA* variants may intensify the downstream signaling, because of their position close to a signal transducer and activator of transcription (STAT)6-recruiting domain.³⁴ Recently, a regulatory cofactor, called STAT6B because of its considerable homology to STAT6, was found to interact with lipopolysaccharide (LPS)-induced TNF- α , the two proteins consequently forming a complex that subsequently translocates into the nucleus and upregulates the transcription of cytokines, including TNF- α .³⁵ The *TNFA* -308G>A could also account for an additional risk of IgE-mediated allergy to betalactams, through this new trans-regulation pathway of *TNFA* that depends on LPS stimulation. An apparently paradoxical result was the association of the -308G>A polymorphism with the presence of specific IgE but not with skin tests (with the exception of the minor determinant). In fact, the positive skin testing is not only related to the production of specific IgE, but also to mast cell and basophile reactivity.⁴ The patients who had positive skin test to minor determinants were at increased risk of cross-reacting reactions between penicillins and cephalosporins.³⁶ It was therefore possible that the *TNFA* polymorphism influences the risk of cross-reactivity. However, in our study, the number of cases with skin tests positive to minor determinant was too small to validate this hypothesis (Table 1).

In conclusion, *TNFA* -308G>A polymorphism is associated with the risk of immediate allergic reaction against betalactams. Our results showed an ambivalent influence of *TNFA* -308G>A, the most frequent allele being associated with the primary risk and the less frequent allele with the

production of specific IgE. The mechanisms that underlie this ambivalent influence could be related to CMH antigen presentation and to the trans-regulation of *TNFA* gene, respectively. Experimental studies are therefore needed to investigate the role of *TNFA* in drug allergy and IgE production.

Materials and methods

Study design and subject enrolment

A total of 169 patients were recruited prospectively from a large outpatient population with a history of immediate reactions to at least one betalactam. This population was evaluated between January 1995 and June 2003 in the Allergy Units of CI Columbus (Rome, Italy) and Oasi Maria SS (Troina, Italy). The patients had reacted to penicillins or cephalosporins and presented an IgE-mediated hypersensitivity to these betalactams diagnosed on the basis of positive responses to skin tests and/or serum-specific IgE assays. We have previously described the clinical and biological characteristics of most of the patients of the present series in two recent articles.^{36,37} The protocol was approved by the local ethics committee. All subjects received information about the possible risks of allergologic tests, and a written informed consent was obtained from each patient or parents of those under 18 years of age. The control group consisted of 265 age- and gender-paired volunteers who were recruited by an allergologist in a preventive care consultation in Roma, on the basis of absence of personal and family history of allergy, including drug allergies (including immediate and nonimmediate reactions), dermatological or respiratory diseases related to allergy and inflammatory diseases. In addition, subjects with a C-reactive protein blood concentration higher than 5 mg/l were excluded. All the patients and controls were of Caucasian origin.

Genotyping of *TNFA* -308 polymorphism

We improved a sensitive method for allowing the determination of the *TNFA* variant with nanogram amounts of DNA isolated from serum. Genomic DNA was isolated from 200 μ l of serum sample using the QuiAmp blood mini kit (Quiagen, Courtaboeuf, France). The genotype of *TNFA* -308G>A polymorphism was determined by real time PCR (Light-Cycler, Roche Molecular Biochemicals, Lyon, France). The anchor (Flu) and detection probes (LC) were labeled at the 3' ends with fluorescein and at the 5' ends with LC-Red-640, respectively. They covered the mutation sites and were modified at the 3' ends by phosphorylation to avoid extension (Tib MolBiol Syntheselabor GmbH, Berlin, Germany). The pair of probes hybridized template DNA with a 1-bp gap between them. PCR was carried out in a 10- μ l mixture in the Light-Cycler glass capillaries. The reaction mixture consisted of 2 μ l of Light-Cycler DNA Master Hybridization Probes (Fast-start *Taq* DNA polymerase, reaction buffer, dNTP mixture and 10 mM MgCl₂; Roche Molecular Biochemicals) and 0.8 μ l of 25 mM MgCl₂. PCR primers were added to the mixture at a concentration of 0.5 μ M, fluorescein-labeled probes at 0.2 μ M and LC-labeled

probes at 0.4 μ M, respectively. Two microliters of DNA solution (20 ng/ μ l) and PCR grade water were then added to the mixture to make a final volume of 10 μ l. The PCR primers, Fu and FC primers were respectively: 5' CCTGCATCCTGTCTGGAAGTTA 3', 5'CTGCACCTTCTGTCTCGGTTT 3' and 5' AACCCCGTCCCCATGCCCC 3', CAAAACCTATTGCCTCCATTCTTTTGGGGAC 3'. The thermal cycling step was as follows: initial denaturation at 95°C for 30s, followed by 40 cycles of denaturation at 95°C for 5s, annealing at 66°C for 10s and extension at 72°C for 10s. The ramp rate was set at 20°C/s. After amplification was completed, melting curves were obtained by holding at 95°C for 5s and then at 52°C for 10s, followed by heating slowly at 0.2°C/s to 95°C with continuous collection of fluorescence at 640 nm. The melting curves were converted to melting peaks by plotting the negative derivative of the fluorescence with respect to temperature (dF/dT) against temperature.

Detection of serum-specific IgE

Blood samples were collected when patients were examined and sera were stored immediately at -20°C until assayed. Assays for serum-specific IgE to penicilloyl G, penicilloyl V, ampicilloyl, and amoxicilloyl (CAP-FEIA) were performed according to the manufacturer's instructions with UniCAP (Pharmacia, Milan, Italy) in all patients. A positive result (that is, detectable specific IgE antibodies) was defined as a value ≥ 0.35 kU/l. Cephalosporin-specific serum IgE were detected by a RIA. We systematically performed RIAs (sepharose-RIA) with a solid-phase drug obtained by coupling cephalosporins to sepharose epoxy-activated 6B (Amersham Pharmacia Biotech Europe, Orsay, France) through a spacer arm, as described previously.^{37,38} The level of serum-specific IgE was determined by measuring the binding of the patient's IgE to the compound, which was covalently bound to epoxy-activated beads, and the results were expressed by means of the binding of the radio-active anti-human IgE antibody.^{37,38} We calculated the percentage of the anti-IgE antibody bound to the solid-phase drug. We also performed the IgE-RIA with all the cephalosporin-sepharose in 40 sera of healthy, nonatopic individuals. The values were reported as the ratio of the percentage bound in the patients to the average percentage bound in the control subjects, measured in the same assay series, as described previously^{37,38} sera with a ratio higher than two were considered positive. Each series of assays was validated by including positive and negative control samples.

Statistical analysis

The statistical analysis was designed to test the hypothesis of an association between *TNFA* -308 polymorphism and the primary risk of immediate allergic reactions to betalactams and the serum levels of specific and total IgE. The frequencies of alleles, genotypes and categorical parameters were expressed as percentages and 95% exact confidence intervals (95% CI). A χ^2 -test was used to assess percentage differences. Continuous variables were reported as means $\pm$ s.d., median, quartiles and range, and compared

by the Mann-Whitney *U*-test. Because the distributions of values for total IgE and specific IgE were skewed, these variables were logarithmically transformed before performing the multivariate analyses. The significance and odds ratios of categorical variables were determined by stepwise multivariate logistic regression analysis, using a residual model that included the variables that were associated with the dependent determinant with $P < 0.15$. A final *P*-value lower than 0.05 was considered to indicate residual statistical significance. The minimal size of our sample was estimated at 100 patients, with a study power $1 - \beta = 0.8$ and $\alpha = 0.05$, assuming a 1.5-fold difference in the less frequent alleles between controls and patients. Data were collected and analyzed using the SPSS 10.0 software for Windows (SPSS, Paris, France).

Acknowledgments

This study was supported by Institutional grants from the Region of Sicily (Italy) and from the University hospital Center of Nancy.

Duality of Interest

The study sponsor had no involvement in study design, data collection, analysis or interpretation, writing of the paper, or the decision to submit the paper for publication.

References

- 1 Cars O, Molstad S, Melander A. Variation in antibiotic use in the European Union. *Lancet* 2001; **357**: 1851–1853.
- 2 Kelkar PS, Li JT. Cephalosporin allergy. *N Engl J Med* 2001; **345**: 804–809.
- 3 Gruchalla RS. Drug allergy. *J Allergy Clin Immunol* 2003; **111**: S548–S559.
- 4 Sicherer SH, Leung DY. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. *J Allergy Clin Immunol* 2005; **116**: 153–163.
- 5 Kalesnikoff J, Baur N, Leitges M, Hughes M R, Damen J E, Huber M et al. SHIP negatively regulates IgE + antigen-induced IL-6 production in mast cells by inhibiting NF- κ B activity. *J Immunol* 2002; **168**: 4737–4746.
- 6 Marquardt DL, Walker LL. Dependence of mast cell IgE mediated cytokine production on nuclear factor- κ B activity. *J Allergy Clin Immunol* 2000; **105**: 500–505.
- 7 Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF- κ B activity. *Annu Rev Immunol* 2000; **18**: 621–623.
- 8 Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA et al. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxine. *Nature* 1984; **312**: 724–729.
- 9 Thomas PS, Pennington DW, Schreck RE, Levine TM. Lazarus SC authentic 17 kD tumor necrosis factor α is synthesised and released by canine mast cells and up-regulated by stem cell factor. *Clin Exp Allergy* 1996; **26**: 710–718.
- 10 Thomas PS. Tumor necrosis factor- α : the role of this multifunctional cytokine in asthma. *Immunol Cell Biol* 2001; **79**: 132–140.
- 11 Suryaprasad AG, Prindiville T. The biology of TNF blockade. *Autoimmun Reviews* 2003; **2**: 346–357.
- 12 McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNF- α promoter region associated with susceptibility to cerebral malaria. *Nature* 1994; **371**: 508–510.
- 13 Cabrera M, Shaw MA, Sharples C, Williams H, Castes M, Convit J et al. Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. *J Exp Med* 1995; **182**: 1259–1264.

- 14 Nadel S, Newport MJ, Booy R, Levin M. Variation in the tumor necrosis factor-alpha gene promoter region may be associated with death from meningococcal disease. *J Infect Dis* 1996; **174**: 878–880.
- 15 Witte JS, Palmer LJ, O'Connor RD, Hopkins PJ, Hall JM. Relation between tumour necrosis factor polymorphism TNF alpha -308 and risk of asthma. *Eur J Hum Genet* 2002; **10**: 82–85.
- 16 Shin HD, Park BL, Kim LH, Jung JH, Wang HJ, Kim YJ et al. Association of tumor necrosis factor polymorphisms with asthma and serum total IgE. *Hum Mol Genet* 2004; **13**: 397–403.
- 17 Gentile DA, Doyle WJ, Zeevi A, Howe-Adams J, Trecki J, Skoner DP. Association between TNF-alpha and TGF-beta genotypes in infants and parental history of allergic rhinitis and asthma. *Hum Immunol* 2004; **65**: 347–351.
- 18 Pirmohamed M, Lin K, Chadwick D, Park KB. TNFa promoter region gene polymorphisms in carbamazepine hypersensitive patients. *Neurology* 2001; **56**: 890–896.
- 19 Wilson AG, Symons JA, McDowell TL, McDevitt H, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997; **94**: 3195–3199.
- 20 Louis E, Franchimont D, Piron A, Gevaert Y, Shaaf-Lafontaine N, Roland S et al. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol* 1998; **113**: 401–406.
- 21 Perri F, Piepoli A, Bonvicini C, Gentile A, Quitadamo M, Di Candia M et al. Cytokine gene polymorphisms in gastric cancer patients from two Italian areas at high and low cancer prevalence. *Cytokine* 2005; **30**: 293–302.
- 22 Albuquerque RV, Hayden CM, Palmer LJ, Laing IA, Rye PJ, Gibson NA et al. Association of polymorphisms within the tumor necrosis factor (TNF) genes and childhood asthma. *Clin Exp Allergy* 1998; **28**: 578–584.
- 23 Winchester EC, Millwood IY, Rand L, Penny MA, Kessling AM. Association of the TNF-alpha-308 (G>A) polymorphism with self-reported history of childhood asthma. *Hum Genet* 2000; **107**: 591–596.
- 24 Wang TN, Chen WY, Wang TH, Chen CJ, Huang LY, Ko YC. Gene-gene synergistic effect on atopic asthma: tumor necrosis factor-alpha -308 and lymphotoxin-alpha-NcoI in Taiwan's children. *Clin Exp Allergy* 2004; **34**: 184–188.
- 25 Moffat MF, James A, Ryan G, Musk AW, Cookson WO. Extended tumor necrosis factor/HLA-DR haplotypes and asthma in an Australian population sample. *Thorax* 1999; **54**: 757–761.
- 26 Westphal GA, Schnuch A, Moessner R, König IR, Kranke B, Hallier F et al. Cytokine gene polymorphisms in allergic contact dermatitis. *Contact Dermatitis* 2003; **48**: 93–98.
- 27 Beghe B, Padoan M, Moss CT, Barton SJ, Holloway JW, Holgate ST et al. Lack of association of HLA class I genes and TNF alpha -308 polymorphism in toluene diisocyanate-induced asthma. *Allergy* 2004; **59**: 61–64.
- 28 Buckova D, Holla LI, Vasku A, Znojil V, Vacha J. Lack of association between atopic asthma and the tumor necrosis factor alpha -308 gene polymorphism in a Czech population. *J Investig Allergol Clin Immunol* 2002; **12**: 192–197.
- 29 Chen G, Wilson R, Wang SH, Zheng HZ, Walker JJ, McKillop JH. Tumor necrosis factor alpha (TNF-a) gene polymorphism and expression in pre-eclampsia. *Clin Exp Immunol* 1996; **104**: 154–159.
- 30 Wilson AG, de Vries N, Pociot F, di Giovine FS, van der Putte LB, Duff GW. An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8 and D3 alleles. *J Exp Med* 1993; **177**: 557–560.
- 31 Romano A, De Santis A, Romito A, Di Fonso M, Vennuti A, Gasbarrini GB et al. Delayed hypersensitivity to aminopenicillins is related to major histocompatibility complex genes. *Ann Allergy Asthma Immunol* 1998; **80**: 433–437.
- 32 Kroeger KM, Steer JH, Joyce DA, Abraham LJ. Effects of stimulus and cell type on the expression -308 tumour necrosis factor promoter polymorphism. *Cytokine* 2000; **12**: 110–119.
- 33 Guéant-Rodriguez RM, Romano A, Béri-Dexheimer M, Viola M, Gaeta F, Guéant JL. Gene-Gene Interactions of IL4RA and IL13 variants in immediate allergic reactions to betalactam antibiotics. *Pharmacogenetics* 2006; **16**: 713–719.
- 34 Zurawski SM, Vega Jr F, Huyghe B, Zurawski G. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. *EMBO J* 1993; **12**: 2663–2670.
- 35 Tang X, Marciano DL, Leeman SE, Amar S. LPS induces the interaction of a transcription factor, LPS-induced TNF-alpha factor, and STAT6(B) with effects on multiple cytokines. *Proc Natl Acad Sci USA* 2005; **102**: 5132–5137.
- 36 Romano A, Guéant-Rodriguez RM, Viola M, Pettinato R, Guéant JL. Cross-reactivity and tolerability of cephalosporins in patients with immediate hypersensitivity to penicillins. *Ann Intern Med* 2004; **141**: 16–22.
- 37 Romano A, Guéant-Rodriguez RM, Viola M, Amoghly F, Gaeta F, Nicolas JP et al. Diagnosing immediate reactions to cephalosporins. *Clin Exp Allergy* 2005; **35**: 1234–1242.
- 38 Guéant JL, Mata E, Monin B, Moneret-Vautrin DA, Kamel L, Nicolas JP et al. Evaluation of a new reactive solid phase for radioimmunoassay of serum specific IgE against muscle relaxant drugs. *Allergy* 1991; **46**: 452–458.