

Genetic restrictions in olive pollen allergy

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Background: The major antigen of olive tree pollen, Ole e 1, produces an IgE response restricted by DQ2.

Objective: Our purpose was to further analyze the genetic restrictions associated with IgE and IgG antibodies against Ole e 1 and IgE against the recently described antigen Ole e 3.

Methods: Twenty-two nuclear olive pollen-allergic families (n = 88) were selected. DRB1 and DQB1, TCR-V α 8.1, the high-affinity receptor of IgE (Fc ϵ RI- β) Rsa I exon 7 and intron 2 and TNF- β (LT α -Nco I) polymorphisms were determined by PCR and analyzed for association with allergic traits by the multiallelic transmission disequilibrium test.

Results: Significant associations were found among HLA-DQB1*0201 (n = 29) and high levels of IgG (P = .023) and IgE (P = .0136) antibodies to Ole e 1 and with IgE specific to Ole e 3 (P = .0368). DRB1*0701 was associated with high levels of total serum IgE (P = .04) and IgG against Ole e 1 (P = .025). The Fc ϵ RI- β Rsa I exon 7, allele 1 (n = 39), was associated with high levels of total serum IgE (P = .01), IgE antibodies against *Olea europaea* extract (P = .004), and specific antibodies to Ole e 1, IgG (P = .04), and IgE (P = .006). The Fc ϵ RI- β Rsa I intron 2, allele 2 (n = 33), was associated with IgE antibodies to *O europaea* extract (P = .003) and specific antibodies to Ole e 1, IgG (P = .025), and IgE (P = .05).

Conclusions: We found a new association between IgE antibody response to Ole e 3 and DQB1*0201 and verified the previously reported association between Ole e 1-specific response and DQB1*0201. Also, the association between Fc ϵ RI- β and IgE antibodies against Ole e 1 was demonstrated. (J Allergy Clin Immunol 2000;105:292-8.)

Key words: Olive pollen allergy, Ole e 1, Ole e 3, HLA class II, IgE high-affinity receptor, T-cell receptor and TNF polymorphisms

In the Mediterranean area and in California, the olive tree (*Olea europaea*) is widely distributed and its pollen is one of the most important causes of respiratory allergy.¹ The major allergen of this pollen, Ole e 1, is a protein of 18 to 20 kd molecular mass,^{2,3} of a heterogeneous

Abbreviations used

DARIA:	Double-antibody RIA
dNTP:	Deoxyribonucleoside triphosphate
Fc ϵ RI- β :	Chain of the high-affinity IgE receptor
LT:	Linfotoxin
MgCl ₂ :	Magnesium chloride
(NH ₄) ₂ SO ₄ :	Ammonium sulfate

nature, that has been cloned and the complete amino acid sequence described.^{4,5} Ole e 1 is an acidic protein that exhibits 2 variants of the same 145-residue polypeptide chain, glycosylated (20 kd) and nonglycosylated (18.5 kd). This protein has at least four B-cell epitopes⁶ defined by mapping with 6 mAbs and 2 regions around 91-102 and 109-130 amino acids have been defined as immunodominant T-cell epitopes.⁷ Finally, the IgE antibody response against this allergen is restricted by DR7-DQ2 antigens^{8,9} demonstrated by genetic associations and by restriction of the response of Ole e 1 T-cell line.

Besides Ole e 1, several IgE-binding proteins have been detected in the saline extract of this pollen,^{2,10} and some of them have been purified and characterized: Ole e 2¹¹ is an olive-pollen profilin with a molecular mass of 15 kd and a recognition frequency estimated at 24% of olive-hypersensitive patients; Ole e 3¹² is a protein that consists of a single polypeptide chain of 9.2 kd with a Ca⁺⁺-binding motif, highly conserved in pollen grains; and Ole e 4 and 5¹³ are 2 allergens purified from olive pollen collected in California. Ole e 4 has an apparent molecular weight of 32 kd (without homology with other known proteins) and an IgE binding frequency of 80%. Recently, another group¹⁴ has identified a 36-kd protein with 83% reactivity that could be the same protein as Ole e 4. Ole e 5 has a molecular weight of 16 kd and a high degree of homology with superoxide dismutase of several plants and a recognition frequency of 35%, and finally Ole e 6¹⁵ is a protein with an apparent molecular mass of 10 kd and an acidic isoelectric point of 4.2.

Asthma and allergies are complex diseases involving genetic as well as environmental factors. The immunogenetic mechanisms underlying heightened IgE responsiveness seen in atopic diseases may be divided into 2 types, antigen-specific and nonantigen specific.¹⁶⁻¹⁸

Strong candidate genes of atopy have been proposed to be located on chromosomes 11q13¹⁹ and 5q31.¹²⁰ where high-affinity IgE Fc receptor β subunit (Fc ϵ RI- β)

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and allergy-associated cytokines (IL-4 gene cluster), respectively, have been mapped. Other candidate genes have been localized on chromosome 12q13.2-q23.3,^{21,22} where the genes of IFN- γ (T_H1 cytokine), stem cell factor, and insulin-like growth factor-1 required for the maturation of mast cells and differentiation of both B and T lymphocytes are located. Also, NFYB (β subunit of nuclear factor-Y, which up-regulates the transcription of IL-4 and HLA-D genes) and BTG1 (B-cell translocation gene 1, which negatively regulates cell proliferation) map in this region.

Genetic regulation of IgE responses to specific allergens may differ from that of the general atopic response. Probably genetic associations are more readily detected with reactions to purified major allergens rather than complex allergen sources. In this respect, mainly 2 chromosomes have been studied, chromosome 6p21.3 and chromosome 14q11.2. On chromosome 6p21.3 genes that encode by MHC class II molecules (HLA-D), which play a pivotal role in the binding and presentation of antigenic peptides to T cell, are located. Several associations with specific IgE antibody response and some HLA class II polymorphisms have been described.^{8,23-27} Other interesting genes located on this chromosome are TNF and linfoxin (LT) α and β genes (potent proinflammatory cytokine that is found in increased concentration in asthmatic airways and in lavage fluid from asthmatic lungs²⁸). Finally chromosome 14q11.2, where the T-cell receptor α chain locus is located, has been associated with specific IgE antibody response.²⁹

In this study we have determined the frequency of response in olive pollen-allergic patients derived from nuclear families against 2 allergens of this pollen, Ole e 1 and Ole e 3, analyzing some of the possible genetic restrictions implicated in this response; as the atopy genes we studied the Fc ϵ RI- β exon 7 and intron 2 polymorphisms on chromosome 11, as genes influencing specific IgE response to particular allergens we typed the DR and DQ antigens on chromosome 6 and the V α 8.1 polymorphisms on chromosome 14, and finally as the gene influencing non-IgE-mediated inflammation we studied the polymorphisms of LT α gene on chromosome 6.

METHODS

Study population

Twenty-two Spanish nuclear families comprising 88 individuals were recruited from the Allergy Department at Hospital General de Especialidades Ciudad de Jaen (Jaen, Spain) according to the HLA and Allergy Workshop (1995) criteria: 2 olive pollen-allergic sibs and at least one of the parents was nonallergic. Informed consent was obtained from all subjects before enrollment in the study. Clinical assessment was done by family and personal physical examination. Immediate hypersensitivity to a battery of the most common allergens (including *O europaea* crude extract) was determined by skin testing. A positive control with histamine (1 mg/mL) and a negative control with a saline buffer solution were also performed for each of the subjects. Peripheral blood was obtained from all subjects (n = 88) for serologic and DNA studies.

Antibody measurement

Total serum IgE levels were measured by Pharmacia IgE enzyme immunoassay (Uppsala, Sweden). Specific *O europaea* extract IgE antibodies were determined by Pharmacia UNI-CAP system (Uppsala, Sweden).

Specific IgG and IgE antibodies to highly purified Ole e 1 allergen were measured by double-antibody RIA (DARIA) as previously described.⁸

Specific IgE antibodies to highly purified Ole e 3 were determined by ELISA.

Antigen purification

Ole e 1 was obtained after gel filtration and HPLC purification by reverse-phase chromatography of crude olive pollen extract (Allergon, Angelholm, Sweden) according to a previously described method.³⁰

Ole e 3 was obtained according to the method described by Ledesma et al.¹¹

Molecular analysis

Genomic DNA was extracted from peripheral blood according to the salting-out method.³¹

The genomic DNA of all individuals was genotyped for 6 polymorphisms: DRB1 and DQB1 HLA class II loci, the V α 8.1 polymorphism³² by analysis of the biallelic *Ava*II polymorphisms, within the Fc ϵ RI- β gene (biallelic *Rsa* I polymorphism in intron 2³³ and biallelic polymorphism in the UTR of exon 7³⁴), and the TNF- β polymorphisms analyzing the biallelic *Nco* I polymorphism in the first intron of the lymphotoxin α gene.³⁵

Polymorphism of the DRB1 locus was studied by reverse dot-blot, according to the manufacturer's indications (Amplicor), and polymorphism of the DQB1 locus was determined by PCR-SSO, according to methods previously described.³⁶

Typing of the TCR V α 8.1 was performed in 2-step PCR reactions as describe Cornélis et al,³² with minor modifications. The first amplification was done in a final volume of 25 μ L with 200 ng of genomic DNA, 0.5 μ mol/L of each primer, 200 μ mol/L of each deoxyribonucleoside triphosphate (dNTP), 67 mmol/L TRIS-hydrochloric acid, 16 mmol/L ammonium sulfate ($[(\text{NH}_4)_2\text{SO}_4]$), 0.01% Tween-20, 2 mmol/L magnesium chloride (MgCl_2), and 0.75 units of Taq DNA polymerase. Amplification conditions were 95°C for 5 minutes followed by 30 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. The second PCR round was in a final volume of 15 μ L, with 1 μ L of 1:100 dilution of the first PCR round and 0.5 μ mol/L of each primer, 200 μ mol/L of each dNTP, 67 mmol/L TRIS-hydrochloric acid, 16 mmol/L $(\text{NH}_4)_2\text{SO}_4$, 0.01% Tween-20, 2 mmol/L MgCl_2 , and 0.75 units of Taq DNA polymerase used as a template. The PCR conditions were 95°C for 5 minutes followed by 14 cycles of 95°C for 1 minute, 56°C for 1 minute, and 72°C for 1 minute.

After amplification, 5 μ L of PCR product was digested with 5 U of *Ava*II (New England Biolabs) at 37°C for 1 hour. Resultant products were analyzed on 2% agarose gels; it was possible to identify 3 genotypes: 1,1 homozygote (124 and 145 bp), 1,2 heterozygote (269, 145, and 124 bp), and 2,2 homozygote (269 bp). Controls for each genotype were included with each set of digests.

For detection of the *Rsa* I polymorphisms within intron 2 and the untranslated region of exon 7 of Fc ϵ RI- β , a specific PCR was carried out, followed by restriction digestion of the PCR products. *Rsa* I exon 7 polymorphisms were determined as described by Palmer et al,³⁴ and *Rsa* I intron 2 polymorphisms were done according to Shirakawa et al.³³ Digestion of the exon 7 with *Rsa* I enzyme gave rise to 3 genotypes: a single 481-bp band denoted the 1,1 genotype; a 481-bp, 295-bp, and 187-bp band pattern denoted the 1,2 genotype; and a 295-bp and 187-bp band pattern denoted the 2,2 genotype.^{34,37}

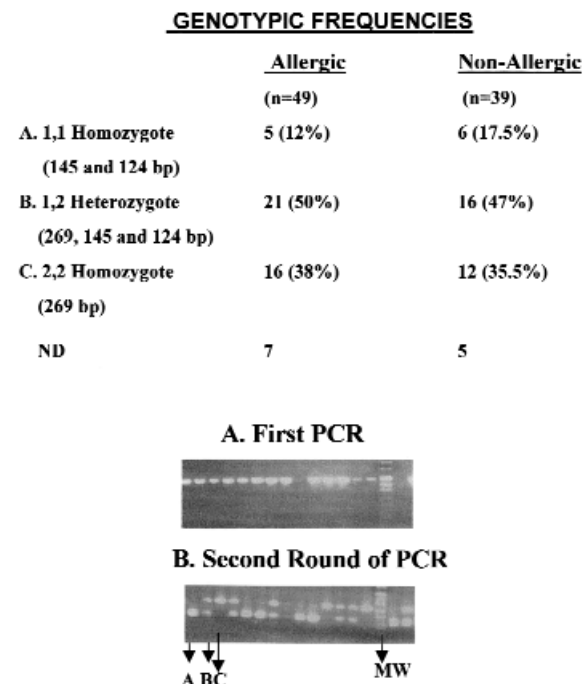


FIG 1. TCR V α 8.1 polymorphisms. Genotypic frequencies distribution in olive pollen-allergic and nonallergic groups. Bottom of figure shows example of agarose gel used for determining polymorphisms, as explained in Methods. **A**, 1,1 Homozygote. **B**, 1,2 Heterozygote. **C**, 2,2 Homozygote. **MW**, Molecular weight markers.

Restriction digestion of the intron 2 PCR product yielded 3 genotypes with an additional constant 148-bp band present as a result of the primers used. The 1,1 genotype was denoted by the presence of an 800-bp and a 148-bp band pattern; the 2,2 genotype by the presence of a 500-bp, a 280-bp, and a 148-bp band pattern; and the 1,2 genotype by an 800-bp, a 500-bp, a 280-bp, and a 148-bp band pattern.

PCR of the LT α NcoI polymorphism was carried out with use of the primers 5'-CCG TGC TTC GTG CTT TGG ACT A-3' and 5'-AGA GCT GGT GGG GAC ATG TCT G-3', generating a 750-bp product. Two hundred nanograms of genomic DNA was amplified in a 15- μ L reaction volume containing 0.5 μ mol/L of each primer with 200 μ mol/L of each dNTP, 67 mmol/L TRIS-hydrochloric acid, 16 mmol/L (NH₄)₂SO₄, 0.01% Tween-20, 1 mmol/L MgCl₂, and 0.45 units of Taq DNA polymerase. Amplification conditions were 95°C for 6 minutes followed by 30 cycles of 95°C for 1 minute, 64°C for 1 minute, and 72°C for 1 minute. A final extension of 72°C for 5 minutes was included. After amplification, 5 μ L of PCR product was digested with 5 U of Nco I (New England Biolabs) at 37°C for 1 hour. Resultant products were analyzed on 2% agarose gels. LT α NcoI allele 1 was identified by 250- and 500-bp fragments and allele 2 by a single 750-bp band. Controls for each genotype were included with each set of digests.

Statistical analysis

Multiple regression analysis was used to examine quantitative traits (total IgE, specific IgE to whole extract, specific IgE and IgG to Ole e 1).

A multiallelic transmission disequilibrium test³⁸ was used to examine associations with qualitative traits (affection status for "high" titers of IgE to Ole e 1 and 3 and high IgG titers to Ole e 1).

Statistical significance of phenotypic or genotypic frequencies between groups was measured by Fisher's exact test.

RESULTS

Patients

Twenty-two nuclear families ($n = 88$) were studied for olive pollen reactivity by skin test. A total of 48 subjects were reactive to *O europaea* extract (in each family, 2 sibs were reactive to *O europaea* extract and at least one of the parents was negative). The population studied, analyzed according to the olive pollen reactivity condition, was composed of 39 nonallergic subjects (45 ± 5 years, mean age) and 49 allergic (16 ± 9 years, mean age).

Antibody results

All the serologic measurements were statistically increased in the allergic group. The mean sera level of total IgE was significantly higher in the group of allergic (580 ± 470 IU/mL) compared with the nonallergic (125 ± 218 IU/mL) patients. Significant levels of IgE antibodies to *O europaea* crude extract (4.5 ± 1.3 UNI-CAP class) were found in the 49 allergic patients and in only 2 in the nonallergic group (0.1 ± 0.44).

Specific IgG antibodies against Ole e 1 were determined in 49 allergic subjects ($31,143 \pm 14,315$ AU/mL), and from these 48 patients (97.9% of the olive pollen-allergic patients) demonstrated significant levels of IgE against Ole e 1 ($10,539 \pm 7546$ AU/mL). Two subjects from the nonallergic group had significant IgG anti-Ole e 1 antibody levels (2322 ± 4770 AU/mL).

Seventeen patients (34.6% of the olive pollen-allergic patients) had significant IgE antibody levels against Ole e 3 (all these patients demonstrated significant IgE antibodies anti-Ole e 1, 0.41 ± 0.64 optical density units).

Genetic analysis

HLA class II polymorphism. The HLA class II results showed that the presence of the allele DRB1*0701 ($n = 19$ allergic subjects) was significantly associated with the total serum IgE levels ($P = .04$) and with high levels of IgG antibodies against Ole e 1 ($P = .025$).

The presence of the DQB1*0201 allele ($n = 29$) was significantly associated with high levels of IgG ($P = .023$) and IgE ($P = .013$) antibodies against Ole e 1, as well as with high antibody levels of IgE to Ole e 3 ($P = .036$).

TCR V α 8.1 polymorphisms. The genotypic distribution for the 3 genotypes analyzed was similar in the allergic and nonallergic groups (Fig 1).

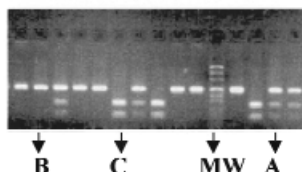
There was no statistically significant association between the polymorphisms analyzed and the allergic traits studied.

Fc ϵ RI- β polymorphisms. There were no statistically significant differences in the genotypic frequencies of the Rsa I polymorphisms studied (exon 7 and intron 2) between allergic and nonallergic groups (Figs 2 and 3).

However, analysis by multiallelic transmission disequilibrium test showed that there were statistically significant associations between allele 1 of exon 7 Fc ϵ RI- β

GENOTYPIC FREQUENCIES

	<u>Allergic</u> (n=49)	<u>Non-Allergic</u> (n=39)
A. 1,2 Heterozygote (481, 295 and 187 bp)	24 (53%)	17 (45%)
B. 1,1 Homozygote (481 bp)	15 (34%)	11 (29%)
C. 2,2 Homozygote (295 and 187 bp)	6 (13%)	10 (26%)
ND	4	1



ASSOCIATIONS

<u>total IgE</u>	<u>α-Olea europaea IgE</u>	<u>α-Ole e 1 IgG</u>	<u>α-Ole e 1 IgE</u>
0.014	0.004	0.006	0.04

FIG 2. *Rsa* I exon 7 *FcεRI* polymorphisms. Genotypic frequencies distribution in patients allergic and not allergic to olive pollen and association between allele 2 and total serum IgE, specific antibody response to olive pollen, analyzed by multiallelic transmission disequilibrium test. **A**, 1,2 Heterozygote. **B**, 1,1 Homozygote. **C**, 2,2 Homozygote. **MW**, Molecular weight markers.

Rsa I polymorphism (n = 39) and total serum IgE levels ($P = .014$) as well as with IgE antibody levels against *O. europaea* extract ($P = .004$) and with IgG ($P = .006$) and IgE ($P = .04$) antibody levels against Ole e 1 (Fig 2).

The same analysis revealed that allele 2 of the *Rsa* I intron 2 *FcεRI*- β polymorphism (n = 33) was significantly associated with the high levels of IgE antibody levels to *O. europaea* extract ($P = .003$) and with the high levels of IgG ($P = .025$) and IgE antibodies to Ole e 1 ($P = .05$) (Fig 3).

TNF- β (LT α -Nco I) polymorphisms. There was no significant difference in the genotypic frequencies observed in the allergic and nonallergic frequencies (Fig 4).

Analysis by a multiallelic transmission disequilibrium test comparing the different allergic traits and the alleles studied did not reveal any statistically significant association.

DISCUSSION

The current study shows the complexity of the allergy response to olive pollen extract. Our results confirm the hypothesis that there are multiple genetic influences in olive pollen allergy.

We have analyzed the response against Ole e 1 and Ole e 3, two well-characterized antigens of olive pollen extract. First, this work confirms by family studies the previous data reported in 2 populations with different genetic backgrounds (Spanish and Arabic subjects) that associated the

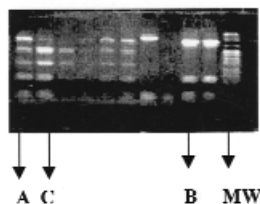
IgE antibody response to Ole e 1 with DR7-DQ2 antigens.^{8,9} Here, the presence of DRB1*0701 was significantly associated with high levels of IgG antibodies to Ole e 1 and DQB1*0201 with IgG α -Ole e 1 and IgE α -Ole e 1.

In spite of the low number of patients with significant levels of IgE α -Ole e 3 (n = 17), the presence of DQB1*0201 was statistically associated with the IgE antibody response to Ole e 3. This similarity in genetic restrictions could indicate similarity at a molecular level between Ole e 1 and Ole e 3, but sequence homology has not been reported.^{4,5,11}

A link between IgE responses to major allergens and the TCR A/D locus has been reported²⁹ and germline HLA-DR and TCR-A interaction in restricting the response to exogenous antigen has been demonstrated.³⁹ Our purpose was to analyze if there was any association between V α 8.1 polymorphism and the specific IgE response against olive pollen allergens. We did not find any association between these polymorphisms and the antigenic responses studied. This result is in accordance with the findings reported by Moffatt et al³⁹ that described the interaction between V α 8.1 polymorphism and HLA-DRB1*1501 in patients allergic to *Der-matophagoides*. Our HLA class II associations (with IgE α -Ole e 1 and Ole e 3) are related with other alleles (DRB1*0701 and DQB1*0201) because it is reasonable to assume that the TCR polymorphism implicated in our specific response could be different.

GENOTYPIC FREQUENCIES

	<u>Allergic</u> (n=49)	<u>Non-Allergic</u> (n=39)
A. 1, 2 Heterozygote (923, 490, 286 and 147 bp)	22 (51%)	14 (41.2%)
B. 1, 1 Homozygote (923 and 147 bp)	10 (23.5 %)	11 (32.5%)
C. 2, 2 Homozygote (490, 286 and 147 bp)	11 (25.5%)	9(26.4 %)
ND	6	5

**ASSOCIATIONS**

	<u>IgEα -Olea europaea</u>	<u>IgG α -Ole e 1</u>	<u>IgE α -Ole e 1</u>
Allele 2	0.003	0.025	0.05

FIG 3. *Rsa* I intron 2 *FcεRI-β* polymorphisms. Genotypic frequencies distribution in patients allergic and not allergic to olive pollen and association between allele 2 and specific antibody response to olive pollen, analyzed by multiallelic transmission disequilibrium test. **A**, 1,2 Heterozygote. **B**, 1,1 Homozygote. **C**, 2,2 Homozygote. *MW*, Molecular weight markers.

GENOTYPIC FREQUENCIES

	<u>Allergic</u> (n=49)	<u>Non-Allergic</u> (n=39)
A. 1,1 Homozygote (500 and 250 bp)	3 (7%)	0
B. 1,2 Heterozygote (250, 500 and 750 pb)	15 (35%)	11 (33.3%)
C. 2,2 Homozygote (750 pb)	25 (58%)	22 (66.7%)
ND	6	6

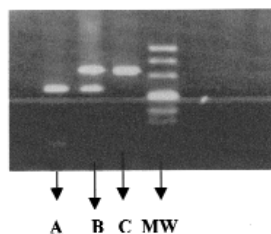


FIG 4. *TNF-β* (*Nco* I *LT-α*) polymorphisms. Genotypic frequencies distribution in patients allergic and not allergic to olive pollen. **A**, 1,1 Homozygote. **B**, 1,2 Heterozygote. **C**, 2,2 Homozygote. *MW*, Molecular weight markers.

Analysis of 2 noncoding *Rsa* I polymorphisms (exon 7 and intron 2) within the *FcεRI-β* gene showed that there are significant associations between this gene and the antibody response observed in olive-allergic patients. We found that allele 1 of *FcεRI-β* *Rsa* I (exon 7) is associated with total serum IgE levels as well as with *O. europaea*-specific serum IgE levels and with Ole e 1-specific IgG and IgE levels (Fig 2). *FcεRI-β* *Rsa* I (intron 2) was significantly associated with *O. europaea*-specific serum levels and with Ole e 1-specific IgG and IgE antibody levels (Fig 3).

These results are in accordance with several reports^{19,34,37,40-43} that describe linkage between coding and noncoding polymorphisms within the β chain of the high-affinity receptor and the serum total IgE, skin prick test responses to allergens, bronchial hyperreactivity, asthma, and atopic dermatitis. However, recently Castro et al⁴⁴ described a lack of association between atopy and *Rsa* I polymorphism within intron 2 of the *FcεRI-β* gene in a Spanish population. Like our results (Fig 3) these authors did not find differences either in the genotypic frequency distribution of atopic and nonatopic populations, concluding that there is no association between these polymorphisms and atopy. However, in contrast with our work, no other allergic traits were studied to

determine possible associations (total IgE, radioallergosorbent assay, specific IgE).

Our results on the association between IgG and FcεRI-β gene are not surprising because it has been described that this chain is common between FcεRI and FcγRIII (low-affinity IgG receptor), suggesting a certain role for IgG antibodies in the elicitation and regulation of anaphylactic reactions.⁴⁵⁻⁴⁷

Finally, we analyzed the Nco I polymorphisms in the lymphotoxin alfa gene as a gene influencing non-IgE-mediated inflammation. Allele 1 of an Nco I polymorphism in the LT α gene has been associated with the level of TNF secretion,³⁵ and this proinflammatory cytokine is increased in asthmatic airways.⁴⁸

In our study there was no association between the polymorphisms tested and the allergic traits analyzed (Fig 4). This result is in agreement with a work that established an association between the increased secretion of TNF and the HLA-DRB1*03 genotype⁴⁹ because our HLA genetic restriction is not related to this genotype.

In summary, our study emphasizes the importance of characterizing the population to be studied, as well as suggesting the implication of multiple genetic influences in olive pollen allergy response.

This is the first time that olive pollen-allergic families have been analyzed at a genetic level comparing the restriction of response against 2 olive pollen allergens. Future research is required for a better understanding of this complex response to improve the diagnosis and therapy of this disease.

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