

Analysis of Polymorphisms in Olive Pollen Allergy: *IL13*, *IL4RA*, *IL5* and *ADRB2* Genes

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Key Words

Allergy and genetic • Asthma • *ADRB2* polymorphisms • Cytokine-related polymorphisms (*IL13*, *IL4RA*, *IL5*) • Olive pollen allergy

Abstract

Background: Previous results demonstrated that sensitization to specific olive pollen allergens could be related with a different clinical pattern (asthma and/or rhinitis), and that specific patterns of sensitization are regulated by different HLA class II antigens. The authors analyze the possible implication of 7 genetic polymorphisms described as asthma susceptibility genes: *IL13* (C–1112T and R130Q), *IL4RA* (I50V, Q551R), *IL5* (C–746T) and *ADRB2* (Q27E and R16G) in specific olive pollen allergic sensitization. **Methods:** The authors genotyped seven polymorphisms of the *IL13*, *IL4RA*, *IL5* and *ADRB2* genes in 146 patients allergic to olive pollen with seasonal rhinitis/asthma and 50 controls using the polymerase chain reaction-restriction fragment length polymorphism and real-time polymerase chain reaction techniques. **Results:** Two polymorphisms of *IL13* were associated with allergy to olive pollen: the TT genotype of *IL13* C–1112T was decreased (odds ratio, OR = 0.35, $p = 0.006$) whereas the RQ heterozygous genotype of *IL13* R130Q increased in patients

allergic to olive pollen (OR = 3.12, $p = 0.009$). The combined analysis of two *IL4RA* single nucleotide polymorphisms (SNPs) (I50V and Q551R) showed an association with asthma: *IL4RA* V50/Q551 was associated with risk (OR = 2.48, $p = 0.007$) whereas the *IL4RA* V50R551 haplotype was associated with protection (OR = 0.31, $p = 0.003$). **Conclusions:** The *IL13* polymorphisms under study were associated with specific allergy to olive pollen: the *IL13* C–1112T polymorphism as a protective factor and the *IL13* R130Q polymorphism as a risk factor. Interestingly, although single polymorphisms of *IL4RA* are not associated with any phenotype analyzed, the interaction between *IL4RA* I50V/Q551R was strongly associated with the asthma phenotype. *IL13* and *IL4RA* could be relevant markers for allergy to olive pollen and asthma development.

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Introduction

Olive tree pollen is one of the most important causes of respiratory allergy in the Mediterranean area [1]. *Olea europaea* pollen induces mainly nasal and conjunctive symptoms [2] although it may also induce asthma exacerbations in areas with high levels of *O. europaea* pollen

in the atmosphere. In Jaén, a southern region of Spain, there is a very high level of pollen (500–1,000 grains/m³ during the pollination season, with peaks of more than 5,000 grains/m³) and a high prevalence of asthma [3].

To date, at least 20 proteins with allergenic activity have been described in olive pollen [4]. Among them, Ole e 1 is the most frequent sensitizing allergen [5, 6]. Besides Ole e 1, 9 additional allergens have also been isolated and purified from *O. europaea* pollen extract [7], some of which, e.g., Ole e 2 and Ole e 10, are major allergens in areas with high levels of pollen in the atmosphere [8, 9]. Furthermore, our previous studies have demonstrated a strong association between Ole e 10- and Ole e 2-specific sensitization and the bronchial asthma clinical phenotype as well as relevant associations among specific sensitizations and HLA class II antigens: Ole e 2 with DR7-DQ2, and Ole e 10 with DR2(15) antigens [9].

The relationship between HLA class II antigens and allergen-specific antibody responsiveness is one of the most extensively studied fields [10]. We have described several genetic and environmental factors associated with olive pollen allergy [11–13]. However, the genetic regulation of allergic responses is complex, probably not only due to the fact that several genes interact with a strong environmental component but also due to their own interaction.

Genes encoding IL-4 and IL-13 play a key role in IgE regulation and allergic reactions. The switching of immunoglobulin class M to E in activated B lymphocytes is largely influenced by the activation of the IL-4/IL-13 cytokine pathway. On the surface of T cells, the binding of IL-4 and IL-13 is facilitated through a heterodimeric receptor composed of the IL-4 receptor α -chain (IL-4RA) and either the common γ -chain (for binding IL-4 and IL-13) or the IL-13 receptor α -chain (IL-13RA) (exclusively for IL-13 binding) [14].

The *IL13* gene is located on chromosome 5q31, a region linked to asthma phenotypes. Two SNPs of *IL13* have been investigated in relation to asthma. One is located in the promoter region at position –1112, adjacent to the nuclear factor of the activated T cell site and has been associated with allergic asthma [15]. The other is a G→A transition at nucleotide +2044 in the coding region of exon 4, which leads to R130Q amino acid substitution, associated with high total serum IgE levels, atopic dermatitis and asthma [16, 17].

IL4RA, the gene encoding the IL-4RA, is located on chromosome 16p12.1. At least two SNPs in the coding region of the *IL4RA* gene lead to amino acid changes in the receptor [18, 19]. An extracellular variant of the IL-

4RA, i.e. substitution of valine at amino acid 50 for isoleucine, has been associated with atopy and atopic asthma [20]. A variant of the intracellular domain, i.e. substitution of glutamine at position 551 for arginine (Q551R), has been associated with atopy, hyper-IgE syndrome and severe atopic eczema and enhanced signaling through the IL-4RA [19].

Another major factor in Th2 response regulation, located on 5q31.1, is *IL5*. The biological activity of *IL5* is specifically focused on the development, differentiation, recruitment, activation and survival of eosinophils [21]. *IL5* C–746T, in the promoter region of the gene, is the only *IL5* SNP described in the European population [22].

Finally, another interesting gene located on chromosome 5q31–q32 is the *ADRB2* gene. The β_2 -adrenoceptor, a cell surface receptor expressed by airway smooth muscle cells has been implicated in the pathogenesis of asthma. Two SNPs in the *ADRB2* gene gave rise to amino acid substitutions in codon 16, with a replacement of Arg with Gly (R16G), and 27, with Gln instead of Glu (Q27E). Both polymorphisms are known to alter the receptor function in vitro [23, 24] and have been associated with the severity and diagnosis of asthma [25, 26].

With this background, we proposed to analyze the role of genes previously associated with allergy and asthma in the immune response regulation of patients allergic to olive pollen, with an extremely high incidence of asthma. We studied the possible association between *IL13* (C–1112T, R130Q), *IL4RA* (I50V, Q551R), *IL5* C–746T and *ADRB2* (R16G, Q27E) polymorphisms and olive pollen allergy, analyzing the association between single polymorphisms, genetic haplotypes or gene-gene and specific clinical phenotypes (olive pollen sensitization vs. nonallergic, asthma vs. rhinitis, total and specific IgE antibody levels and correlation with IL-13 and IL-5 levels).

Patients and Methods

Subjects

We selected 146 consecutive unrelated patients recruited at the Allergy Service of the 'Complejo Hospitalario de Jaén' who fulfilled the following criteria: seasonal rhinitis and/or asthma from April to June, a positive skin prick test for *O. europaea* pollen extract (ALK Abelló, Madrid, Spain), and no previous *O. europaea* immunotherapy.

Fifty healthy subjects from the same geographic area were recruited as a control group.

Informed consent was obtained from each subject. Ethical approval for skin testing with purified allergens in human subjects was obtained from the Ethical and Research Committee of the hospital.

Table 1. Genotyping conditions for the *IL13*, *IL4RA*, *IL5* and *ADRB2* polymorphisms**a** Genotyping conditions by PCR-RFLP

Gene	Polymorphism	Location	Alternative identifiers	db SNP rs no.*	Primers	PCR condition °C ^a	Restriction enzyme	Allele	Fragment sizes
<i>IL13</i>	C-1112T	promoter	C-1055T	rs1800925	5'-GGAATCCAGCATGCCTTGTGAGG-3' 5'-GTCGCCTTTTCCTGCTCTTCCCGC-3'	54	<i>Bst</i> UI (0.2U)	C T	224 247
<i>IL4RA</i>	I50V	exon 5	Ile50Val	rs1805010	5'-GGCAGGTGTGAGGAGCATCC-3' 5'-GCCTCCGTTGTTCTCAGGTA-3'	60	<i>Rsa</i> I (1U)	A G	254 273
<i>ADRB2</i>	R16G	exon 1	Arg16Gly	rs1042713	5'-CCTTCTTGCTGGCACCCCAT-3' 5'-CCGTCCTGCAGACGCTCGAAC-3'	60	<i>Nco</i> I (2U)	G A	174 157
	Q27E	exon 1	Gln27Glu	rs1042714	5'-GGCCCATGACCAGATCAGCA-3' 5'-GAATGAGGCTTCCAGGCGTC-3'	60	<i>Fnu</i> 4HI (0.4U)	G C	174 229

b Genotyping conditions by RT PCR

Gene	Polymorphism	Location	Alternative identifiers	db SNP rs no.*	Primers	Allele
<i>IL13</i>	R130Q	exon 4	+2044A/G Arg130Gln	rs20541	CCAGTTTGTAAAGGACCTGCTCTTAC TCCTGTCTCTGCAAATAATGATGCT	sense antisense
						AGGGACGGTTCAAC AGGGACAGTTCAAC
<i>IL4RA</i>	Q551R	exon 12	Gln551Arg	rs1801275	GCCGAAATGTCCTCCAGCAT TGCTCCACCGCATGTACAA	sense antisense
						CAGTGGCTATCAGGAGT CAGTGGCTATCGGGAGT
<i>IL5</i>	C-746T	promoter		rs2069812	TTCTCTGCTGCTCATGAACAGAA TCCATCCTTGGGCACCTTTC	sense antisense
						TGAGGACCTAGACATACAT TGAGGACCTAGATATACAT

^a PCR annealing temperature. Polymorphic nucleotides in the allele sequences of *IL13* R130Q, *IL4RA* Q551R and *IL5* C-746T are highlighted. * www.ncbi.nlm.nih.gov/SNP.

Clinical Assessment

Clinical assessment performed in these patients was previously described [9]. Patients recorded symptom scores, drug requirements and peak expiratory flow rates (PEFR) every day from April to June. Individual nasal and eye symptoms (sneezing, blockage, running, redness and itching), and chest symptoms (breathlessness, wheezing and tightness) were recorded on a scale of 0–3 (0 = no symptoms; 1 = mild symptoms; 2 = moderate symptoms; 3 = severe symptoms). The main clinical variable analyzed was defined as an asthma day. An asthma day fulfilled at least one of the following criteria: asthma symptoms score ≥ 2 , morning PEF 20% lower than the mean morning PEF of the last 7 days before pollination, and daytime use of salbutamol twice or more compared with the mean use of the last 7 days before pollination.

Olive Pollen Allergens

Olive tree pollen and olive pollen allergens were isolated as previously described [7, 9].

Total and Specific IgE Antibody Measurements

Total serum IgE levels and specific *O. europaea* extract IgE antibodies were determined by Pharmacia systems (IgE enzyme

immunoassay and UNI-CAP, Uppsala, Sweden). Allergen-specific IgE antibody measurements (Ole e 1, 2, 3, 6, 7, and 10) of allergic sera were performed by enzyme-linked immunosorbent assay (ELISA) [27].

Skin Testing

All 146 patients and 50 controls were tested for olive pollen allergy by a skin prick test with *O. europaea* whole extract and olive pollen purified allergens following the recommendations of the EAACI [28].

The patients were also tested with a panel of common aeroallergens.

Molecular Analysis

Genomic DNA was extracted from peripheral blood using the salting-out method [29].

Genomic DNA of all individuals was genotyped for 7 polymorphisms: *IL13* (C-1112T, R130Q), *IL4RA* (I50V, Q551R), *IL5* (C-746T) and *ADRB2* (R16G, Q27E).

SNPs and their identifier names, locations, alternative names and dbSNP numbers are shown in table 1.

Genotypes for *IL13* C-1112T, *IL4RA* I50V, and *ADRB2* R16G and Q27E polymorphisms were assigned using the polymerase

chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and for *IL13* R130Q, *IL4RA* Q551R and *IL5* C-746T polymorphisms, according to the Applied Biosystems allelic discrimination assay-by-design protocol (AB-7500 Real Time PCR System).

All genotyping details including primers and assay conditions are given in the respective references [17, 24, 30, 31] with minor modifications (table 1).

Measurement of Soluble Cytokine Levels

Levels of soluble IL-5 and IL-13 were analyzed in patient sera by flow cytometry, using the BD Cytometric Bead Array (CBA), human TH1/TH2 Cytokine Kit (Becton Dickinson, BD, San Diego, Calif., USA) for IL-5 and the BD CBA Human Soluble Protein Flex Set System (BD).

Flow-cytometric analysis was performed using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif., USA).

Data were acquired and analyzed by Becton Dickinson (BD) Cytometric Bead Array (CBA) Software.

Statistical Analysis

Comparisons of phenotypic or genotype frequencies between groups (olive-pollen-sensitized vs. controls, asthmatic vs. rhinitis) were measured by Fisher's exact test or by the χ^2 test.

Cochran and Mantel-Haenszel and two-factor ANOVA tests were used for the qualitative and quantitative statistical comparison of clinical parameters, respectively between sensitized and nonsensitized groups and asthmatic vs. rhinitis groups. Multiple-regression analysis was used to examine quantitative traits (total IgE, specific IgE to *O. europaea* whole extract, specific olive pollen allergens IgE and IL-5 and IL-13 serum levels). When multiple comparisons were analyzed, the ANOVA test with the Bonferroni post-hoc contrast was used in order to minimize chance associations.

All SNPs were first analyzed separately and genotype categories were created. Haplotype frequencies for all polymorphisms were estimated using the expectation-maximization algorithm (EM) implemented by Arlequin software (Arlequin v3.01, CMPG, University of Bern, Bern, Switzerland). Statistical analyses of these haplotypes were performed with the EpiInfo v5.00 (CDC, Atlanta, Ga., USA) statistical package

Results

Patients

Clinical features of patients included in the study are summarized in table 2.

The control group included 33 females (66%) and 17 males (34%), with a mean age of 37.6 years (range 23–58 years). They were selected from the same geographical area and were free from any allergic symptoms. They were matched for gender and age. No relevant differences were found.

Table 2. Clinical features of the patients population

Clinical features		
Sex, female/male		
92/54		
Mean age, years		
22.9 $\pm$ 7.1		
Total IgE, IU/ml (medians-interquartile ranges)		
P25: 109 P50: 202 (mean = 401.8 $\pm$ 502) P75: 467		
<i>O. europaea</i> IgE, AU/ml (medians-interquartile ranges)		
P25: 5.5 P50: 22.05 (mean = 36.9 $\pm$ 36) P75: 65		
Atopic diseases		
Bronchial asthma Rhinitis		
109 (74.7%) 37 (25.3%)		
Frequency of recognition, %		Mean specific sera levels, OD/ml (medians-interquartile ranges)
Ole e 1	91.8	P25: 0.114 P50: 0.463 (mean = 0.98 $\pm$ 0.95) P75: 1.765
Ole e 2	70	P25: 0.11 P50: 0.394 (mean = 0.89 $\pm$ 0.79) P75: 1.195
Ole e 3	38.7	P25: 0.26 P50: 1.35 (mean = 0.28 $\pm$ 0.71) P75: 2.5
Ole e 6	50	P25: 0 P50: 0.036 (mean = 1.026 $\pm$ 0.88) P75: 0.85
Ole e 7	52.3	P25: 0 P50: 0.04 (mean = 1.33 $\pm$ 0.95) P75: 1.26
Ole e 10	54.4	P25: 0.04 P50: 0.18 (mean = 0.94 $\pm$ 0.79) P75: 0.78

Olive Allergen Sensitization

All the allergic subjects (n = 146) showed significant IgE antibody levels against pollen crude extract, but different frequencies of sensitization were observed for the 6 purified *O. europaea* allergens (table 3). Ole e 1, Ole e 2, Ole e 7 and Ole e 10 are major allergens in our population (>50% of recognition frequency).

Molecular Analysis

The genotype distribution of the 7 polymorphisms examined was in Hardy-Weinberg equilibrium.

Genotype frequency distribution in the patient and control groups and the genotype distribution of asthma or rhinitis in our patient population are shown in table 3.

Table 3. Genotypic frequency distribution of the *IL13*, *IL4RA*, *IL5* and *ADRB2* polymorphisms

Polymorphism	Genotypes	Patients	Controls	Asthma	Rhinitis	p value	
						controls vs. patients	asthma vs. rhinitis
<i>IL13</i> C-1112T	CC	49 (33.6)	17 (34)	36 (33.0)	13 (35.1)	0.006 OR 0.35 ^a (0.16–0.76)	NS
	CT	71 (48.6)	14 (28)	53 (48.6)	18 (48.6)		
	TT	26 (17.8)	19 (38)	20 (18.3)	6 (16.2)		
<i>IL13</i> R130Q	GG	87 (60.0)	41 (82)	68 (63.0)	19 (51.4)	0.009 OR 3.12 ^a (1.29–7.79)	NS
	GA	54 (37.2)	8 (16)	38 (35.1)	16 (43.2)		
	AA	4 (2.8)	1 (2)	2 (1.9)	2 (54.0)		
<i>IL4RA</i> I50V	AA	45 (30.8)	16 (32)	35 (32.1)	10 (27.0)	NS	NS
	AG	77 (52.7)	23 (46)	52 (47.7)	25 (67.6)		
	GG	24 (16.4)	11 (22)	22 (20.2)	2 (5.4)		
<i>IL4RA</i> Q551R	AA	90 (61.6)	34 (68)	69 (63.3)	21 (56.8)	NS	NS
	AG	50 (34.2)	14 (28)	37 (33.9)	13 (35.1)		
	GG	6 (4.1)	2 (4)	3 (2.8)	3 (8.1)		
<i>IL5</i> C-746T	CC	62 (42.5)	22 (44)	44 (40.4)	18 (48.6)	NS	0.045 OR 0.34 ^{b, c} (0.1–1.15)
	CT	69 (47.3)	22 (44)	57 (52.3)	12 (32.4)		
	TT	15 (10.3)	6 (12)	8 (7.3)	7 (18.9)		
<i>ADRB2</i> R16G	AA	24 (16.6)	8 (16)	17 (15.7)	7 (18.9)	NS	NS
	AG	72 (49.7)	25 (50)	54 (50.0)	18 (48.6)		
	GG	49 (33.8)	17 (34)	37 (34.3)	12 (32.4)		
<i>ADRB2</i> Q27E	CC	62 (43)	24 (48)	49 (45.8)	13 (35.1)	NS	NS
	CG	59 (41)	22 (44)	40 (37.4)	19 (51.4)		
	GG	23 (16)	4 (8)	18 (16.8)	5 (13.5)		

Figures in parentheses are percentages.

^aSignificant p values in comparisons between patients versus controls.

^bSignificant p values in comparisons between asthmatic versus subjects rhinitic allergy.

^cNonsignificant result because 1 is comprised in the CI.

IL13 C-1112T SNP

The association between the *O. europaea* allergy and the *IL13* C-1112T polymorphism was analyzed by comparing the genotype frequencies of this polymorphism between sensitized patients and controls. We found a statistically significant decrease in the TT homozygote genotype in patients compared with controls ($p = 0.006$, odds ratio, OR, 0.35; 95% confidence interval, CI, 0.16–0.76) (table 3). Interestingly, the sera of TT homozygous patients showed the lower levels of soluble IL-13 compared with CT heterozygous and CC homozygous patients, but the differences were not statistically significant (data not shown).

When we analyzed the genotype distribution of this polymorphism according to the clinical phenotype of

asthma or rhinitis and their correlation with total or specific IgE antibody levels, no relevant association was found.

IL13 R130Q SNP

The analysis of genotype frequency distributions of the *IL13* R130Q polymorphism showed a statistically significant increase ($p = 0.009$) in RQ heterozygous patients (37.2%) compared with the control group (16%) (table 3). Patients with this genotype have a more than 3-fold risk to become sensitized to *O. europaea* compared with control subjects (OR, 3.12; 95% 1.29–7.79). As shown above, this genotype (RQ heterozygous) was associated with the highest IL-13 serum levels compared with QQ and RR homozygous.

We also found a statistically significant increase ($p = 0.039$) in the prevalence of the R homozygous genotype in the allergic patients group sensitized to Ole e 3 (71.4%) compared with patients nonsensitized to this allergen (52.8%; OR, 2.23; 95% CI, 1.03–4.86; data not shown). With regard to asthma or rhinitis, no differences were found even for IgE antibody levels.

IL4RA I50V SNPs

We found no statistically significant differences in the distribution of *IL4RA* I50V genotypes between any of the study groups examined (case vs. control, asthma vs. rhinitis, levels of total and specific IgE antibodies against *O. europaea*).

However, the analysis of specific IgE antibody levels against olive pollen allergens showed statistically significant differences ($p = 0.025$) in the homozygous I50 genotype, that were decreased in the Ole e 3 sensitized patients (19.3%) compared with Ole e 3 nonsensitized patients (38.2%). This genotype seems to confer protection against Ole e 3-specific sensitization (OR, 0.39; 95% CI, 0.16–0.9) (data not shown).

Among the patients, we observed that the homozygous V50 genotype was significantly associated ($p = 0.045$) with the highest serum α -*O. europaea* IgE antibody levels (48.35 ± 8.26 IU/ml) compared with both the I50 homozygous genotype (38 ± 5.48 IU/ml) and I50V heterozygous genotype (32.8 ± 4.18 IU/ml) (data not shown).

IL4RA Q551R SNP

We found no statistically significant differences in the distribution of *IL4RA* Q551R genotypes between any of the study groups examined (case vs. control, asthma vs. rhinitis, levels of total and specific IgE antibodies against α -*O. europaea*).

IL5 C-746T SNP

The association between the *O. europaea* allergy and the *IL5* C-746T polymorphism was analyzed by comparing the genotype frequencies of this polymorphism between allergic patients and controls. As shown in table 3, the genotype frequency distribution is similar between cases and controls.

We observed a decrease in the TT genotype in patients with asthma (7.3%, $n = 8$) when compared to patients with rhinitis (18.9%, $n = 7$; $p = 0.045$; OR, 0.34; 95% CI, 0.1–1.15), but as the 1 is included into the CI, this result it is considered nonsignificant. However, according with this result and, although we did not found statistically

significant differences in the soluble IL-5 levels of the patient sera, TT homozygous patients showed the lower levels of soluble IL-5 compared with CT heterozygous and CC homozygous patients (data not shown).

The analysis of the correlation between specific antigen sensitization and genotype distribution showed no statistically significant differences.

According to Ole e 2-specific sensitization, we found that the highest α -Ole e 2 IgE antibody levels were statistically significantly associated ($p = 0.02$) with the TT genotype (1.67 ± 0.31 IU/ml; $n = 10$) when compared with both the CT heterozygous genotype (0.847 ± 0.11 IU/ml; $n = 46$) and CC homozygous genotype (0.746 ± 0.11 IU/ml; $n = 45$) (data not shown).

ADRB2 SNPs

No association was observed between the *ADRB2* R16G and Q27E polymorphisms and the risk of developing olive pollen allergy (table 3). A similar pattern was observed with the clinical phenotype of asthma or rhinitis, total IgE and specific sensitization to *O. europaea* or specific olive pollen allergens.

Haplotype Analysis

In order to investigate the combined effect of SNPs throughout the development of specific olive pollen allergy of our patients, haplotype analysis was also performed. The results of these analyses are shown in figures 1 and 2.

The isolated effects of single alleles of the respective SNPs on the risk of becoming sensitized to olive pollen are shown in the upper panel of figure 1. In a second step, pairs of SNPs of the same gene as well as pairs of SNPs located on the same chromosome were analyzed, and finally, triplets were formed. According to the stepwise introduction of SNPs, the lower risk to become sensitized to olive pollen as a single effect is associated with the R allele of the *IL13* R130Q polymorphism (OR, 0.41; 95% CI, 0.19–0.87, $p = 0.017$) (data not shown).

Analyzing the combined effect of SNPs by *IL13* T-1112/R130 haplotypes or triplets formed by *IL5* T-746/*IL13* T-1112/R130 we did not find any interaction because the OR values for the combinations were similar to those for single alleles.

In a second analysis, the genetic effects on a clinical phenotype (asthma versus rhinitis) were studied according to the same stepwise procedure. Interestingly, an interaction was observed between the two polymorphisms analyzed for *IL4RA*. Figure 2 shows that the OR of an individual polymorphism is similar in asthmatic and non-

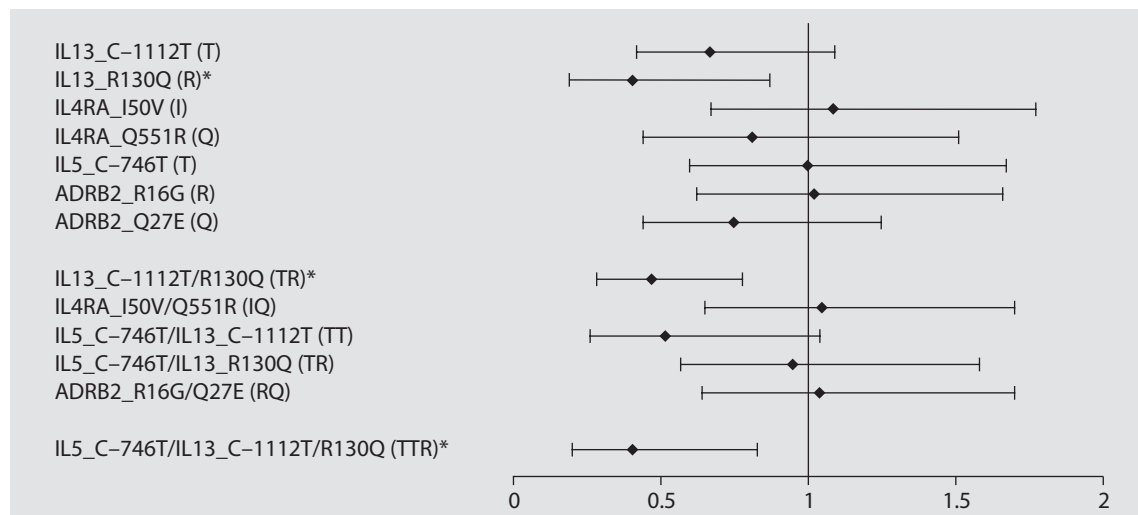


Fig. 1. Association between combinations of *IL13*, *IL4RA*, *IL5* and *ADRB2* alleles and the risk of olive pollen sensitization. Effects of single SNPs are shown in the upper panel, effects of haplotype pairs in the middle panel; the lower panel displays the haplotype triplet. * Statistically significant interactions.

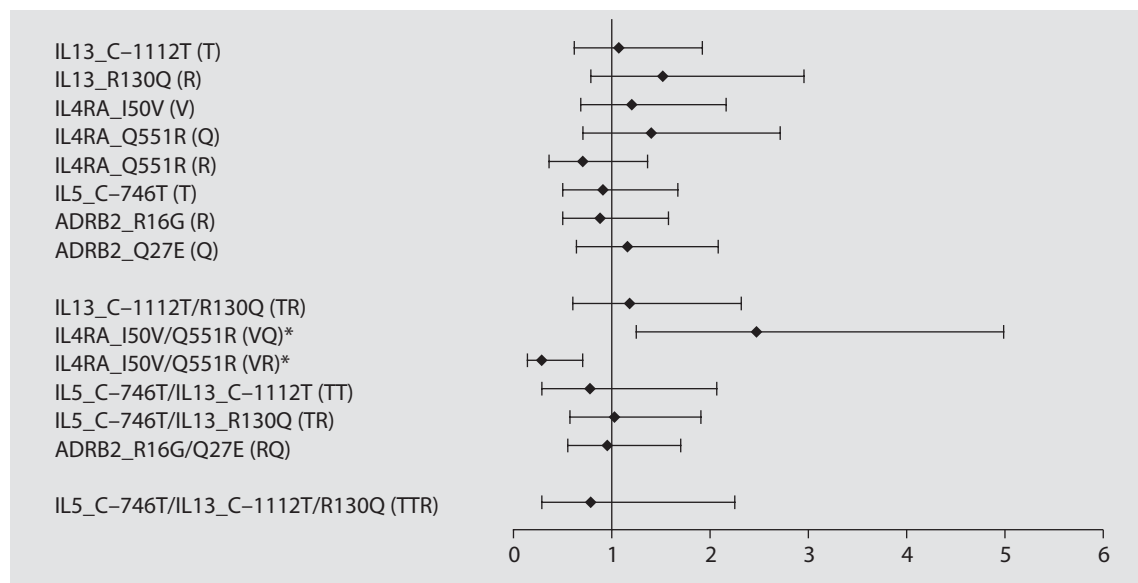


Fig. 2. Association between *IL13*, *IL4RA*, *IL5* and *ADRB2* allele combinations and the risk of asthma. Upper panel: effects of single SNPs; middle panel: effects of haplotype pairs; lower panel: haplotype triplet. * Statistically significant interactions.

asthmatic patients. However, a statistically significant OR was observed in the *IL4RA* I50V/Q551R haplotype. The risk of asthma was increased ($p = 0.007$) for the interaction between *IL4RA* V50/Q551 (OR, 2.48; 95% CI, 1.25–4.99), while the interaction between *IL4RA* V50/R551 decreased the risk ($p = 0.0037$) (OR, 0.31; 95% CI,

0.14–0.71). The two genotypes seem to be a risk and a protective factor, respectively, for the development of a phenotype of asthma in our population.

We found statistically significant differences in haplotype distribution according to specific antigen sensitization. *IL13* C-1112/R130 was increased ($p = 0.034$) in pa-

tients sensitized to Ole e 6 (59%) compared to nonsensitized individuals (46%; OR, 1.69; CI, 1.04–2.76). The same haplotype was also increased in patients sensitized to Ole e 7 (60%) compared with Ole e 7 nonsensitized individuals (46%; OR, 1.77; CI, 1.08–2.9). Therefore, *IL13* T–1112/R130 was decreased in Ole e 7-sensitized patients (20% vs. 31%; data not shown).

IL4RA V50/Q551 significantly increased ($p = 0.0001$) in Ole e 3-sensitized patients (46%) compared with Ole e 3 nonsensitized individuals (24%; OR, 2.72; CI, 1.59–4.65; data not shown).

Finally, we also analyzed the role of the gene-gene interaction of the polymorphisms not localized on the same chromosome. The genetic interactions were studied between the *IL4RA* and *IL13*, and *IL5* and *IL4RA* polymorphisms (data not shown). There were no statistically significant results.

Discussion

Sensitization to specific allergens is a complex response controlled by both genetic and environmental factors. The aim of this work was to analyze the relationship between 7 genetic polymorphisms of *IL13*, *IL4RA*, *IL5* and *ADRB2* and specific sensitization to *O. europaea* pollen in a well-characterized Spanish population of patients with olive pollen allergy and also a high prevalence of asthma (74.7%). This population lives in a region with an extremely high antigenic load, which could be related to a 'special olive pollen sensitization' as is reflected by our results, i.e. 4 of the 6 allergens analyzed are major allergens in this population (Ole e 1, 2, 7 and 10), and not in populations with a lower antigenic exposure. These results are in accordance with a different clinical phenotype as we previously described [8, 9]. These special characteristics together with our previous results [11–13] led us to analyze genetic polymorphisms related mainly to asthma and IgE regulation although the sample size was small.

The SNPs and gene-gene interaction analyses are of interest because they give a global point of view of the mechanisms that determine the allergic response and thus are more informative than single-polymorphism analyses. For this reason, in this report, we first analyzed SNP effects, second SNP interactions and finally, gene-gene interactions.

Given the lack of previously published data on *IL13*, *IL4RA*, *IL5* and *ADRB2* SNPs in subjects with olive pollen allergy, we cannot directly compare our results with

others. However, and in spite of the small size of our control population, several preliminary conclusions can be drawn from our data.

In the present study, we showed that the two polymorphisms of the *IL13* gene analyzed are associated with the genetic regulation of *O. europaea* allergy.

The homozygous TT genotype of the *IL13* C–1112T polymorphism appears to be a protective factor to development of olive pollen allergy. These data are in discordance with those obtained in several studies where both the T allele and TT genotype were associated with allergic asthma [15, 32] and high total serum IgE [17, 33]. These data could suggest that each specific sensitization is controlled by different genetic and environmental factors. In our study, and in spite of the lack of statistical significance, it is very remarkable that there is a correlation between the lower IL-13 serum levels and the *IL13* TT polymorphism, related with the protective factor against sensitization. We think that this result is very promising and should be confirmed in a larger population.

On the other hand, the *IL13* R130Q polymorphism has been associated with high total serum IgE levels [16, 17], atopic dermatitis [16], and asthma [34, 35] in different populations [16, 17, 34] whereas other studies did not find such associations [32, 36]. We found that the R130Q genotype was an important risk factor for *O. europaea* allergy and that it was implicated in specific sensitization to the Ole e 3 olive pollen allergen. Interestingly, and in agreement with the association we established, we found a correlation with the heterozygous genotype and the highest IL-13 levels, but without statistical significance, probably due to the small sample studied.

Neither of the two polymorphisms of the *IL13* gene studied showed any association with the development of some specific clinical phenotype (asthma/rhinitis) or with the IgE levels in our population.

We also analyzed the combined effect of *IL13* SNPs on specific olive pollen sensitization.

According to the epistasis theory, interaction effects between SNPs may exist, notably when several SNPs (with individual effects) are combined, an additive effect could occur and the OR would increase accordingly (additive).

The combined analysis of the *IL13* C–1112T/R130Q and *IL5* C–746T/*IL13* C–1112T/R130Q polymorphisms did not reveal any additive or multiplicative effects, thus we cannot consider any genetic interaction (fig. 1).

According to specific sensitization, the *IL13* C–1112/R130 haplotype was associated with the risk of Ole e 6- and Ole e 7-specific sensitization. On the contrary, the

IL13 T-1112/R130 haplotype conferred protection against Ole e 7-specific sensitization.

As to the *IL4RA* gene, a number of polymorphisms have been identified to be associated with asthma and atopy [37]. In our population, no single genotype of the I50V and Q551R *IL4RA* polymorphisms was related with the development of the *O. europaea* allergy or with a specific clinical phenotype (asthma or rhinitis). Whereas the homozygous I50 genotype of the *IL4RA* I50V polymorphism was associated with protective pattern for Ole e 3-specific sensitization, the V50 genotype was also correlated with the highest serum α -*O. europaea* IgE antibody levels.

In the genetic interaction analysis we found that the individual allelic analysis of its polymorphisms, I50V and Q551R, showed no relevant association. However, the allelic combination of *IL4RA* I50V and Q551R revealed a strong association between the clinical phenotype of asthma and these polymorphisms. This result suggests that the combination of the I50V and Q551R polymorphisms of the *IL4RA* gene have a higher effect on asthma induced by *O. europaea*-specific sensitization than the single polymorphisms of *IL4RA*. The OR value for *IL4RA* V50 was 1.22 and 1.41 for Q551 whereas it was 2.48 for V50/Q551. This interaction was associated with a risk pattern for the development of asthma. The V50/R551 interaction was associated with a protective factor. The OR of *IL4RA* V50/R551 was 0.31, and 0.71 for the single allele *IL4RA* R551. The combined effects of these two polymorphisms exceeded the single effects for each of them. These data mean that a genetic interaction exists between the *IL4RA* I50V and Q551R polymorphisms in our population. This result contradicted that obtained by another group where the V50R551 haplotype is related with atopic asthma [38]. Discrepant results could be attributed to the different characteristics of the populations and different allergen sensitizations. Our patients suffer from asthma induced by a specific sensitization to *O. europaea*-specific allergens whereas the clinical phenotype of asthma in the other population has not been induced by only one specific sensitization. Interestingly, our results are in accordance with a previous report in a Spanish population that described how the A allele (Q genotype) of 576Q > R *IL4RA* polymorphism, together with the T allele of -33C > T-*IL4*, were associated with an increased risk of asthma [39].

Gene-gene interactions have been observed between *IL4RA* and *IL13* with respect to asthma and total serum IgE levels [14, 40–42]. These interaction studies were performed between the combination of both polymorphisms of *IL4RA* and *IL13* in our population. We found no relevant interactions between these polymorphisms.

IL-5 is important in allergic inflammation and some studies suggest the possibility of *IL5* polymorphism being associated with asthma severity but not with asthma development. Our results showed that the *IL5* polymorphism alone was not associated with increased sensitization against olive pollen allergy. However, we found an association between the *IL5* C-746T polymorphism and a decreased of risk of developing asthma. These data are in agreement with a recent report that describes the T allele as a protective factor against atopy and allergic asthma [43]. However, in our study as OR was included between two opposites values of the CI (protection and risk factor), the result should be confirmed with a larger or different population sample in spite of this drawback, the correlation between the TT genotype and the lowest levels of *IL-5* in sera of allergic patients seems very interesting. As described for *IL-13*, our preliminary results of interleukin serum levels in allergic patients are in concordance with our results on polymorphism. This aspect should be studied more in detail in order to confirm or not the possible functionality of these polymorphisms.

Finally, an important controversy exists on the potential contribution of *ADRB2* polymorphisms to the risk of asthma and its prognosis. Several studies demonstrate that genetic variations in the human *ADRB2* at codons 16 and 27 alter receptor function [23, 44] and are associated with asthma severity [45] and airway hyperresponsiveness [46], but they have not been linked to asthma diagnosis, while other groups described an association with asthma [25, 26, 47].

In our own study, we found no association between these genetic variants of *ADRB2* and the risk of olive pollen sensitization and asthma. In agreement with other researchers, we found that the R16 segregated more commonly with Q27 and G16 with E27. These alleles are in strong linkage disequilibrium and the R16/E27 haplotype is most uncommon [48].

This is the first time that these polymorphisms have been studied in a population with *O. europaea*-specific sensitization. Our results showed how the individual analysis of single polymorphisms could supply biased viewpoints. On the other hand, studies of correlation between interleukin levels and polymorphisms should be analyzed in more extended populations in order to confirm possible functional implications.

Our results are a complex reflection of this kind of response, but at the same time, and in spite of the main limitation of our study (small control size), we have defined both risk and protective patterns, which could be useful for both diagnosis and treatment in the future.

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