

Allergy to *Dermatophagoides* in a Group of Spanish Gypsies: Genetic Restrictions

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Key Words

Gypsies · *Dermatophagoides* allergy · HLA class II · Fcε receptor (high-affinity IgE receptor) · Tumor necrosis factor · T cell receptor · Genetic polymorphism

Abstract

Background: Spanish gypsies have traditionally lived as nomads, a reason why few epidemiological studies were done in this ethnic group. However, the high prevalence of asthmatic diseases demonstrated in a population residing in the North of Spain induces us to analyse whether it was due to the influence of genetic loci previously implicated in other population studies as causing the disorders. **Methods:** DRB1* and DQB1* HLA class II, TCR-Vα8.1, FcεRI-β *Rsa* I exon 7 and intron 2, TNF-β (LTα-*Nco*I) and CD14, were tested for association with asthma and atopy by multiple regression analysis, in 5 families comprising 87 individuals. **Results:** Significant associations were found with DQB1*02 ($p = 0.02$) and DQB1*0301 ($p = 0.008$) and elevated levels of total serum IgE. A negative association ($p = 0.02$) was found between total serum IgE and DRB1*14. FcεRI-β *Rsa* I-In2 allele 1

was associated with high levels of total serum IgE ($p = 0.04$). Levels of Der p 1 IgE antibodies were negatively associated with DRB1*11-DQB1*0301 ($p = 0.007$), and positively with TCR Vα-8 allele 1 ($p = 0.04$) and with FcεRI-β *Rsa* I-In2 allele 1 ($p = 0.009$). **Conclusions:** Our results do not show any association between asthma and the genetic loci studied although they do suggest the existence of multiple genetic influences on the allergic response in these families.

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Introduction

Atopy is a genetic predisposition to respond to environmental allergens with significant amounts of specific IgE antibodies. It is associated with different symptomatology ranging from asymptomatic individuals to bronchial asthmatics, allergic rhinitis sufferers and individuals with atopic dermatitis. The variability in the atopic phenotype shows the complexity of these diseases, reflecting the implication of genetic as well as environmental factors.

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1018–2438/01/1254–0297\$17.50/0

Accessible online at:
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The estimated prevalence of atopy, based on a study on 16 countries, ranges from 16% in Spain to 45% in New Zealand with atopy defined as the presence of at least 1 positive specific IgE response [1].

Dermatophagoides pteronyssinus is the predominant house dust mite in many parts of the world, and multiple IgE-binding components have been identified in extracts of these species [2]. Der p 1 and Der p 2 are the major *D. pteronyssinus* allergens and over 80% of asthmatic individuals recognize these allergens in different populations [3, 4]. A recent study on the prevalence of atopy in Spain [5] described *D. pteronyssinus* as the most common allergen in four of the five areas of Spain studied (in the fifth area the most common allergen was olive pollen).

The IgE antibody response to specific allergens (purified major allergen rather than complex allergen sources) has been associated with two chromosomes, 6p21.3 and chromosome 14q11.2. The genes that encode MHC class II molecules (HLA-D) are located on chromosome 6p21.3. These molecules play a pivotal role in the binding and presentation of antigenic peptides to T cells. A great deal of research has been performed in this field, and several associations with specific IgE antibody response and HLA class II polymorphisms have been described [6–8]. The T cell receptor α -chain locus is located on chromosome 14q11.2 which has been associated with specific IgE antibody response [9].

Strong candidate genes of atopy have been mapped to chromosome 11q13 (the high-affinity IgE Fc receptor) [10] by linkage analysis or by association studies and to chromosome 5q31.1 where the cytokine cluster is located [11]. This cluster includes the genes of IL-3, IL-4, IL-5, IL-13, GM-CSF, the glucocorticoid receptor and the β_2 -adrenergic receptor gene. Other candidate genes have been located on chromosome 12q13.2-q23.3 [12], where the genes of interferon- γ (Th1 cytokine), stem cell factor (SCF) and insulin-like growth factor-1 (IGF-1) required for the maturation of mast cells and differentiation of both B and T lymphocytes reside. NFYB (β -subunit of nuclear factor-Y, which upregulates the transcription of IL-4 and HLA-D genes) and BTG1 (B-cell translocation gene 1, which negatively regulates cell proliferation) are also found within the 12q13.2-q23.3.

Recently, Baldini et al. [13] have described the possible implication in the regulation of total serum IgE levels of a CD14 polymorphism, whose gene is interestingly also located on chromosome 5q31.1. This receptor is constitutively expressed on the surface of monocytes, macrophages and neutrophils. It has specificity for lipopolysaccharides and other bacterial-wall-derived components.

Baldini et al. [13] have suggested that variants in the promoter region of the CD14 gene might alter the expression of CD14. This in turn could regulate the proportion of Th2- to Th1-type cells responding to environmental stimuli and thus influencing total serum IgE levels.

The present report tries to demonstrate how the high prevalence of asthma and allergy to mites observed in a population of gypsies may reflect an underlying genetic regulation and is not only due to environmental factors derived from a change in lifestyle. In this work, we analyze a population of Spanish gypsy families established in the North of Spain, in order to identify some of the genetic restrictions implicated in asthma and *Dermatophagoides* allergy. We have studied as atopy genes, the exon 7 and intron 2 polymorphisms of Fc ϵ RI β on chromosome 11. As genes influencing specific IgE response to particular allergens we have typed the HLA-DRB1* and DQB1* antigens on chromosome 6 and the TCR-V α 8.1 polymorphism on chromosome 14. As the gene influencing non-IgE-mediated inflammation, we investigated a polymorphism of the lymphotoxin- α (LT- α) gene on chromosome 6, and, finally, we studied the regulation of total IgE via CD14 polymorphism on chromosome 5.

Materials and Methods

Study Population

Five families (1 extended and 4 nuclear families) comprising 87 individuals (48 women and 39 men) were recruited from the Allergy Department at Hospital Santiago Apóstol and Centro de Salud de Sansomendi (Vitoria, Spain). Informed consent was obtained from all subjects prior to enrollment in the study. Clinical assessment was by family and personal physical examination. Immediate hypersensitivity to a battery of the most common allergens (including *D. pteronyssinus* and *D. farinae* 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 subject. Peripheral blood was obtained from all subjects (n = 87) for serological and DNA studies.

Criteria used for asthma diagnosis were the standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma [14].

Subjects with high levels of total serum IgE, positive skin test and specific IgE antibodies against some of the common allergens were considered as atopic subjects.

Antibodies and Soluble CD14 Measurements

Total serum IgE levels were measured by Pharmacia (Uppsala, Sweden) IgE enzyme immunoassay. Specific *D. pteronyssinus* extract, Der p 1, Der p 2 and Der f 1 IgE antibodies, were determined by the ALK-Abelló (Madrid, Spain) standards ELISA method.

Serum soluble CD14 (sCD14) levels were measured using a commercial ELISA supplied by Biosource (Camarillo, Calif., USA).

Molecular Analysis

Genomic DNA was extracted from peripheral blood following the salting out method [15].

The genomic DNA of all individuals was genotyped for seven polymorphisms: DRB1* and DQB1* HLA class II loci, the biallelic *Ava* II polymorphism within TCR Va8.1 [16], two polymorphisms within the FcεR1-β gene (biallelic *Rsa* I polymorphism in intron 2 [17] and biallelic polymorphism in the UTR [18] of exon 7), the tumor necrosis factor-β polymorphism, analyzing the biallelic *Nco* I polymorphism in the first intron of LTα [19] and the promoter region of the CD14 gene analyzing the biallelic *Ava* II polymorphism [13].

The polymorphisms of the DRB1*, DRB3*, DRB4*, DRB5* and DQB1* loci were studied by amplifications with sequence-specific primers, according to the manufacturer's instructions (Micro-SSP, One Lambda).

Typing of the TCR Va8.1 was performed as described by Cornelis et al. [16], with minor modification [20]. Resultant products of PCR, after digestion with *Ava* II (New England Biolabs), were analyzed on 2% agarose gels and three genotypes were identified: 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 FcεR1β, a specific polymerase chain reaction (PCR) was carried out followed by restriction digestion of the PCR products. Digested products were resolved on a 2% (w/v) agarose gel containing 1 μg/ml ethidium bromide, run for 60 min at 70 V in a BioRad (Hemel, Hampstead, UK) wide subcell. Digestion of the exon 7 polymorphism gave rise to the following three 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 [18].

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

PCR of the LTα *Nco* I polymorphism was carried out using 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. Following amplification, 5 μl of PCR product was digested with 5 U of *Nco* I (New England Biolabs) at 37° for 1 h. Resultant products were analyzed on 2% agarose gels, LTα *Nco* I 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.

Typing of the promoter region of the CD14 gene was performed according to a restriction fragment assay described by Baldini et al. [13]. A 497-bp PCR product encompassing the interval bp -513 to -17 bp was generated using primer 5'-GTGCCAACAGATGAGG-TTCAC-3' and 5'-GCCTCTGACAGTTTATGTAATC-3'. PCR product was digested with *Ava* II endonuclease (New England Biolabs, Beverly, Mass., USA) according to the manufacturer's instructions. The assay thus yields one 497-bp band for the CC genotype, three bands of 144, 353 and 497 bp for CT heterozygotes, and two bands of 144 and 353 bp for TT homozygotes.

Statistical Analysis

For comparison between atopics and nonatopics, the Cochran and Martel-Haensel test and two factors ANOVA test were used.

Linkage was examined using two non-parametric methods (methods that make no assumption about mode of inheritance or allele frequencies, and which are the most appropriate for complex diseases): SAGE and SIMIBD [21, 22]. Both are based on the drawing of associations between the disease (or trait) and the degree of marker allele-sharing between sibs ('sibpair' analysis).

SAGE was used to examine linkage to the affection trait (atopy diagnosis) and the quantitative traits [ln(IgE), IgE α-*D. pteronyssinus* extract, specific IgE against Der p 1, Der p 2 and Der f 1].

SIMIBD is a method specifically designed for examining linkage of a disease trait and markers in extended (general) pedigrees. Unlike SAGE, which looks at sib pairs within an extended pedigree, SIMIBD looks at all affected relative pairs.

SIMIBD was used to examine linkage between disease state (asthma) and each of the genetic markers.

Associations to quantitative traits were analyzed in three ways: (1) Allele-based analysis of variance (using SPSS 6.1), for examining the variation in the trait according to the type of marker allele; (2) Genotype-based analysis of variance on the biallelic markers (using SPSS 6.1), for examining variations in the traits according to the genotype, (3) Multiple regression analysis (using SPSS 6.1), for analyzing the correlation between the different parameters [skin test, RAST, Der p 1 and Der p 2 IgE, ln(IgE) and age] and to examine the effects of these traits and the number of copies of each allele on the variation in the trait.

Statistical significance of allelic or genotypic frequencies between groups was measured by Fisher's exact test or the χ^2 test.

Results

Patients

Eighty-seven subjects derived from 5 families (1 extended and 4 nuclear) were studied. Figure 1 shows the pedigrees of the families analyzed.

Thirty-one subjects were asthmatic (36.9%, with a prevalence ranging from 60% in family 4 to 30.7% in family 3) and 53 were nonasthmatic. Asthma diagnosis was not done in 3 subjects.

After establishing by a statistical approach that there was no difference in the behavior of the different families according to the parameters to be analyzed, the 5 families were analyzed together. When the subjects were classified according to the atopic condition (as defined in Material and Methods), a significant increase in asthmatic subjects was observed in the atopic group compared to the nonatopic group (63.4 vs. 10.8%) (table 1). The prevalence of atopy was not related to sex. The mean age of the atopic group was significantly lower (27 ± 8.7 years) than that of nonatopic subjects (35 ± 19 years). The number of subjects with reactivity to *D. pteronyssinus*, as determined by skin tests, was statistically higher in the atopic group (65.8%) compared with the nonatopic group (17%). The mean value of reactivity was also significantly higher in

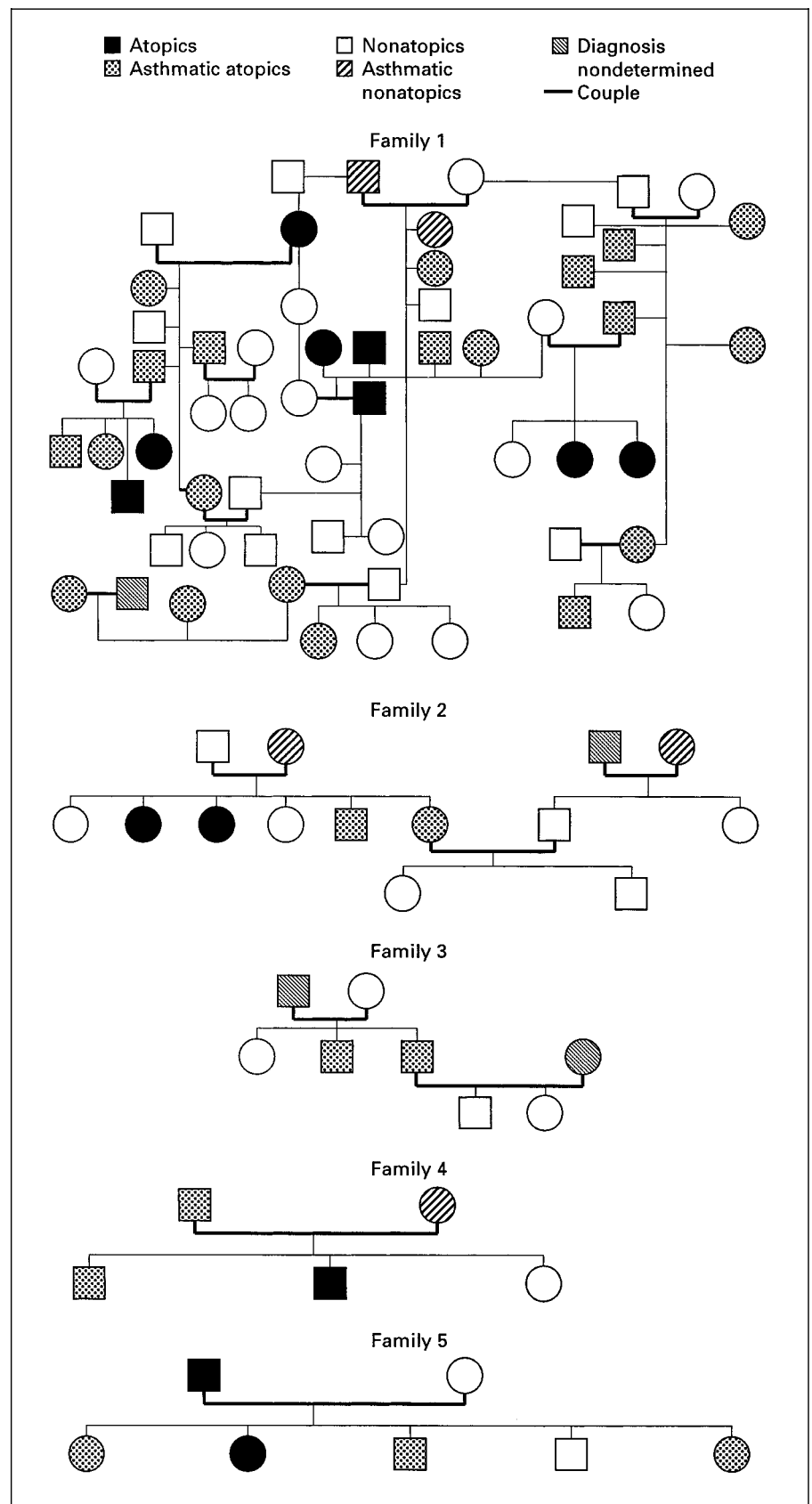


Fig. 1. Pedigree charts of the five families studied. Boxes represent males and circles females.

the atopic group (8 ± 3 mm) than in the nonatopic group (5.2 ± 1.7 mm) ($p < 0.01$) (table 1).

Antibody Results

All the serological measurements were significantly higher in the atopic group compared to the nonatopic group (table 1). The mean serum level of total IgE was significantly higher in atopics (597 ± 751 IU/ml) compared to nonatopics (61 ± 55 IU/ml).

A high percentage of atopic patients ($n = 40$, 97.5%) showed significant levels of IgE antibodies to *D. pteronyssinus* crude extract with a mean value of 48 ± 33 IU/ml. In contrast, only 1 subject from the nonatopic group (2.4%) showed IgE antibodies to *Dermatophagoides* and the mean serum IgE value in this group (0.7 ± 4.2) was significantly lower ($p < 0.01$).

Specific IgE antibodies against Der p 1, Der p 2 and Der f 1 were found in both groups of patients. A high percentage of atopic patients showed significant levels of IgE antibodies against all three allergens. The percentage of reactivity was significantly increased in atopics versus nonatopics for each allergen: Der p 1 (80.4 vs. 6.9%), Der p 2 (85.3 vs. 4.6%) and Der f 1 (46.3 vs. 4.6%) (table 1).

sCD14 Measurements

The sCD14 levels were similar in atopics (5.7 ± 2.6 µg/ml) and nonatopics (5.4 ± 2.6 µg/ml). No statistically significant correlation was found between sCD14 levels and other allergic traits analyzed.

Genetic Analysis

Analysis of the Locus Implicated in Specific Responses

HLA Class II. The frequencies of the HLA class II antigens DR and DQ were analyzed comparing the results obtained in atopic versus nonatopic groups. In addition, we analyzed the differences between our two study groups and the phenotypic frequencies described by de Pablos et al. [23] in healthy Spanish Gypsies (considered as a control population for this ethnic group). The results are summarized in table 2. DRB1*14 was the most prevalent antigen in the study population: 25 of 41 atopic subjects (60.9%) having this antigen and 33 from 46 individuals (71.7%) in the nonatopic group. Statistically significant differences were found in the presence of this antigen when nonatopics were compared with healthy Spanish gypsies (71.7 vs. 50.6%, $p = 0.02$).

DRB1*13 was significantly decreased ($p = 0.01$) in the atopic group (no subject showed this antigen) compared with the control population (15.6%).

Table 1. Clinical parameters

	Atopics (n = 41)	Nonatopics (n = 46)	p
Females, %	48.7	57.7	
Age, years	27 ± 8.7	35 ± 19	0.02
Asthmatics	n = 26 (63.4)	n = 5 (10.8)	<0.01 ¹
Total IgE, IU/ml	597 ± 751	61 ± 55	<0.01
<i>Dermatophagoides</i>	n = 27 (65.8)	n = 8 (17)	<0.01 ¹
Skin test, mm	8 ± 3	5.2 ± 1.7	<0.01
<i>D. pteronyssinus</i>	n = 40 (97.5)	n = 1 (2.4)	<0.01 ¹
Extract IgE, IU/ml	48 ± 33	0.7 ± 4.2	<0.01
Der p 1 IgE, CAP	n = 33 (80.4)	n = 3 (6.9)	<0.01 ¹
Class units	3.3 ± 0.98	0.9 ± 0.9	<0.01
Der p 2 IgE, CAP	n = 35 (85.3)	n = 2 (4.6)	<0.01 ¹
Class units	3.3 ± 1.1	0.7 ± 0.9	<0.01
Der f 1 IgE, CAP	n = 19 (46.3)	n = 2 (4.6)	<0.01 ¹
Class units	2.2 ± 1.2	0.8 ± 0.9	<0.01

Figures in parentheses are percentages.

¹ Comparison performed by two-factor ANOVA test.

The frequency of the DQB1*02 antigen was significantly increased in the atopic group (46.3%) compared with the nonatopic group (23.9%) with a p value of 0.04, and was also increased when compared with the Spanish Gypsy control group (22.3%), with a p value of 0.01. Due to the limited number of patients under study, none of the results were statistically significant after correcting the p value in accordance with the number of comparisons made.

Significant results of association by multi-allelic transmission disequilibrium test between allergic traits and the different HLA class II antigens studied are summarized in table 3. The presence of DQB1*02 and DQB1*0301 was significantly associated with high levels of total serum IgE antibodies ($p = 0.02$ and 0.008 , respectively). However, the presence of DRB1*14 was significantly associated with low levels of total serum IgE antibodies ($p = 0.02$).

The DRB1*11-DQB1*0301 haplotype was significantly associated with low levels of specific IgE antibody against Der p 1 ($p = 0.007$).

TCR Vα8.1. The distribution for the three genotypes analyzed was similar in the atopic and nonatopic groups: 1,1 homozygotes (24.3% atopics vs. 17.4% nonatopics), 1,2 heterozygotes (48.7 vs. 50%) and 2,2 homozygotes (21.9 vs. 26%). Two atopics (4.8%) and 3 nonatopics (6.5%) were not typed (data not shown).

Table 2. HLA frequencies and TCR allelic frequencies: comparison between the atopic and nonatopic groups

Marker	Allele	Spanish gypsies, % (n = 75) ¹	Atopics (n = 41)	Nonatopics (n = 46)	p value	Corrected p value
HLA class II DRB1*	DRB1*11	20.8	12 (29.2)	8 (17.3)	NS	NS
	DRB1*13	15.6	0 (0)	2 (4.3)	0.01 ²	NS
	DRB1*14	50.6	25 (60.9)	33 (71.7)	0.02 ³	NS
HLA class II DQB1*	DQB1*0301	26.3	12 (29.2)	8 (17.3)	NS	NS
	DQB1*0201	22.3	19 (46.3)	11 (23.9)	0.04 ⁴	NS
TCR Vα8.1	Allele 1	ND	30 (73.1)	31 (67.3)	NS	
	Allele 2	ND	29 (70)	35 (76)	NS	

ND = Not determined; NS = nonsignificant.

¹ Frequencies described by de Pablos et al. [23] in a population of healthy Spanish gypsies.

² Statistically significant difference between healthy Spanish gypsies and atopic gypsies.

³ Statistically significant difference between healthy and nonatopic Spanish gypsies.

⁴ Statistically significant difference between atopic and nonatopic Spanish gypsies.

Table 3. Association between allergic traits and genetic loci by multiple-regression analysis

Allergic traits	Positive association	Negative association
Total IgE serum levels	DQB1*02 (p = 0.02) DQB1*0301 (p = 0.008) FcεRIβ <i>Rsa</i> I-in2 allele 1 (p = 0.04)	DRB1*14 (p = 0.02)
Specific Der p 1 IgE antibody levels	TCR Vα8.1 allele 1 (p = 0.04) FcεRIβ <i>Rsa</i> I-in2 allele 1 (p = 0.009)	DRB1*11 (p = 0.007) DQB1*0301 (p = 0.007)

Frequencies for allele 1 and 2 were similar in both groups (table 2). However, the presence of allele 1 was significantly associated (p = 0.04) with high specific IgE antibody levels against Der p 1 (table 3).

Analysis of the Results of the Locus Implicated in Atopy: FcεRIβ

There were no significant differences in the genotypic frequencies of the *Rsa* I polymorphisms studied (FcεRI-β *Rsa* I-in2 and FcεRI-β *Rsa* I-ex7) between atopic and nonatopic groups. However, the presence of allele 2 of FcεRI-β *Rsa* I-in2 was significantly lower (p = 0.007) in the atopic group (46.3%) compared with nonatopics (60.8%) (table 4).

Analysis by the multi-allelic transmission disequilibrium test showed that there were significant associations between allele 1 of FcεRI-β *Rsa* I-in2 and total serum IgE antibody levels (p = 0.04), as well as with specific IgE antibody levels against Der p 1 (p = 0.009) (table 3).

Gene Influencing Non-IgE-Mediated Inflammation: TNF-β (LT α-*Nco* I) Polymorphisms

There was no significant difference in the genotypic frequencies observed in the atopic and nonatopic groups: 1,1 homozygote (0% atopics vs. 2.1% nonatopics), 1,2 heterozygote (12.1 vs. 17.3%) and 2,2 homozygotes (73.1 vs. 36.9%). Genotypes were not available for 14.6% of the atopics and 21.7% of the nonatopics. The allele frequencies were similar in atopics and nonatopics (table 4).

Table 4. Comparison of allelic frequencies of FcεRI-β (intron 2 and exon 7) and TNF-β polymorphisms, between atopic and nonatopic subjects

Marker	Allele	Atopics (n = 41)	Nonatopics (n = 46)	p value
FcεRIβ	allele 1	39 (95.1)	43 (93.4)	NS
<i>Rsa</i> I-in2	allele 2	19 (46.3)	28 (60.8)	0.007
FcεRI-β	allele 1	33 (80.4)	35 (76)	NS
<i>Rsa</i> I-ex7	allele 2	34 (82.9)	33 (71.7)	NS
LT-α <i>Nco</i> I	allele 1	5 (12.1)	9 (19.5)	NS
	allele 2	35 (85.3)	35 (76)	NS

Figures in parentheses are percentages.

Analysis by multi-allelic transmission disequilibrium test correlating the different allergic traits and the alleles studied did not reveal any statistically significant associations.

CD14

The genotypic distribution for the three genotypes analyzed (n = 67) was similar in the atopic and nonatopic groups: CC homozygotes (16.2% atopics vs. 13.3% non-atopics), CT heterozygotes (59.5% vs. 63.3%) and TT homozygotes (24.3 vs. 23.3%).

There was no correlation between the different allergic traits and the polymorphisms analyzed; however, TT individuals (n = 16) had lower total serum IgE levels (209 ± 408 IU/ml) compared with CC (n = 10, 452 ± 800 IU/ml) and CT (n = 41, 291 ± 551 IU/ml) individuals.

The sCD14 levels showed the following distribution: CC (6.68 ± 1.9 µg/ml), CT (5.6 ± 2.5 µg/ml) and TT (5.1 ± 3.1 µg/ml). No statistical differences were found.

Discussion

Asthma and atopy arise from interactions between strong genetic and environmental factors. Great progress is being made in this field finding linkage between asthma-associated traits and specific chromosomal loci [24]. Interestingly, some of these genes or gene families are relevant in a variety of inflammatory and immune diseases, and are not exclusive to asthma and atopy.

In previous studies, the associations between atopy, asthma and dwelling conditions in a gypsy population liv-

ing in the district of Sansomendi (14,000 inhabitants) in the North of Vitoria (Spain) were analyzed [25]. The sample was composed of 266 gypsies attending the outpatient's clinic who were compared with 271 nongypsies from the same location. An increase of asthma and atopy was found among the gypsy population. With respect to asthma, the prevalence was five times higher and positive skin tests against one or more allergens were found in 39.7% of gypsies versus 24% in nongypsies. Total IgE was also increased in the gypsy population: 293 versus 122 IU/ml in nongypsies. To assess if this high prevalence is related with dwelling factors, an epidemiological study was performed analyzing the humidity, temperature, and allergenic exposure to Der p 1 [26]. The differences found in the characteristics of both types of housing of gypsies and nongypsies did not explain the prevalence of the disease. It is well known that some other factors may be implicated in allergy and for this reason we studied five families (one extended and four nuclear) at the genetic level in the search for an association between allergy and specific genetic loci in this population.

Several immunogenetic studies have been performed in mite-sensitive asthmatic patients and these have shown the complexity of this kind of response. Although some reports describe a lack of association between HLA and the response to *Dermatophagoides* [27, 28], the linkage between the HLA-D region and the Der-p-specific IgE has been demonstrated in Caucasian families [29, 30]. Several associations have been found: HLA-DRB1*0101, DPB1*0401-02, 0501 as implicated in the restriction of Der p 1 peptides [31], whereas the Der p 2 antibody response has been associated with HLA-DRB1*1101 [32]. Recently, an association of DRB1*1101 DQA1*0501 DQB1*0301 has been related with *Dermatophagoides*-sp.-sensitive asthma in a Venezuelan population [33]. Also, in a Chinese population an association between HLA-DQw2 and mite-reactivity was found in asthmatic children [34].

As far as we know, few studies have been carried out on the gypsy population [23, 35], emphasizing the complexity of this type of research. In relation to the Spanish phenotypic frequencies for the different antigens studied, de Pablo et al. [23] described a significant increase in DR14-DQ5 whilst DQ2 was decreased. In this respect, it is interesting that antigen DQ2 is one of the most prevalent in the Spanish population as a whole. Results from Pablo et al. [23] indicated that Spanish gypsies are closer to Indian Caucasoids than to the Spanish nongypsy population. In our study, DRB1*14 is the most prevalent antigen and there is a negative association between this anti-

gen and total IgE serum levels ($p = 0.02$, table 3). However, the fact that the frequencies of this antigen are similar in atopic and nonatopic subjects rules out the possibility that this antigen is a protective factor. Probably, other genetic and nongenetic factors are implicated in the sensitization.

Table 2 shows an increase of DQ2 in atopic gypsies (46.3 vs. 22.3 and 23.9% in healthy and nonatopic gypsies, respectively). This antigen, with a minor representation in healthy individuals from this ethnic group, is positively associated with the total IgE serum levels ($p = 0.02$, table 3), indicating a possible role of this antigen as a risk factor for atopy. Similar results have been obtained by Gerbase-De Lima et al. [35] in asthmatic children with a frequency of DQ2 of about twice the values obtained for nonasthmatics (relative risk: 2.8).

Between gypsies we found similar DQB1*0301 frequencies in the three studied groups (table 4). However, a positive association was found with DQB1*0301 and total IgE serum levels ($p = 0.008$, table 3).

Specific IgE levels against Der p1 were found to be negatively associated with the haplotype DRB1*11-DQB1*0301 ($p = 0.007$, table 3). This same haplotype has been described by Lara et al. [33] to be positively associated with asthma due to *Dermatophagoides* in a Venezuelan population and there was no association between specific IgE antibodies and these antigens. By contrast, in our population no association was found with asthma and HLA class II, probably due to the selection of an allergic population and the different genetic background.

A link between IgE responses to major allergens and the TCR A/D locus has been reported [9] and a restriction of the response to exogenous antigen has been demonstrated [37] in relation with germline HLA-DR and TCR-A. Our purpose was to analyze if there was any association between the V α 8.1 polymorphism and the specific IgE response to *Dermatophagoides* in our population. We found an association between TCR V α 8.1 allele 1 and specific IgE Der p 1 response ($p = 0.04$, table 3) but not with Der p 2 and Der f 1 (data not shown). In this regard, Moffat et al. [37] described the association between specific IgE antibody response to Der p 2 and V α 8.1 allele 2 in HLA-DR2 (15) patients. These results agree with the idea that HLA and TCR are important but are not the only genetic factors also implicated in this response. Probably, non-HLA IgE-regulating loci and environmental factors also contribute to the expression of overall IgE responsiveness toward the Der p allergens in addition to HLA-D genes [29].

Good candidate genes, located on chromosome 6 are the tumor necrosis factor (TNF) and LT α and β genes,

potent proinflammatory cytokines that are found in increased concentration in asthmatic airways and in lavage fluid from lungs of asthmatic patients [38].

We analyzed the *Nco* I polymorphism in the LT α gene as a gene influencing non-IgE-mediated inflammation. Allele 1 of the *Nco* I polymorphism has been associated with the level of TNF secretion [19] and this proinflammatory cytokine is increased in asthmatic airways. However, we did not find any linkage between asthma and the genetic inflammatory locus studied (table 4).

The analysis of two non-coding *Rsa* I polymorphisms (intron 2 and exon 7) within the Fc ϵ RI β gene revealed significant associations between this gene and the antibody response in *Dermatophagoides*-allergic patients. We found allele 1 of Fc ϵ RI β *Rsa* I-in2 to be positively associated with total serum IgE levels ($p = 0.04$) as well as with specific IgE anti-Der p 1 ($p = 0.009$). In accordance, the frequency of allele 2 was significantly decreased in atopics ($p = 0.007$, table 4). No associations were seen with Fc ϵ RI β *Rsa* I-ex7.

These results agree with several previous studies [17, 18, 39] 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.

It has been suggested that bacterial infections may play a role in promoting the balance between Th1/Th2 patterns. Consequently, CD14 is a potentially important receptor which is expressed on the surface of monocytes, macrophages and neutrophils. Few studies have been done in relation to the Th1/Th2 balance. Due to the special background of our population, we decided to analyze the CD14 polymorphism as a possible regulator of the Th1/Th2 response. Our results do not reflect any involvement of this molecule in the allergic parameters analyzed in our study population. Baldini et al. [13] studied a population of different ethnic composition and they did not find a correlation between TT genotype and low levels of total serum IgE or positive skin test in some of the ethnic groups (i.e. among Hispanic children there was no association). Our results may be due to the fact that our population did not show any dwelling differences when compared with nongypsies living in the neighborhood [25]. Nevertheless differences may be found if we compare this population with those living in nonsedentary conditions.

In summary, this is the first report relating allergy and genetic traits in a Spanish gypsy population. The present data reflect the complexity of allergic response, implicating a number of genetic loci in this disorder. Further

research, increasing the number of subjects and the genetic loci, studying different ethnic groups and carried out in different environmental conditions is required to enable better understanding of this universal health problem.

Acknowledgments

We thank Mr. Steven Wiltshire (Asthma and Genetics Group Oxford, UK) for the statistical analysis and Drs. D. Barber and J. Moscoso (ALK, Abelló, Madrid, Spain) for specific IgE determina-

tions. We acknowledge the continued support of the European Science Foundation, network Programme on Immunogenetics of Allergy (ESF). B. Cárdbaba was the recipient of a travel grant from ESF at the Asthma and Genetics Group in Oxford (UK).

Also, we appreciate the support given by Fundación Lair to Ignacio Arrieta, and by the Fundación Conchita Rábago to Marta Rojo. We acknowledge the technical assistance of P. Tramón (ATL).

This work was supported in part by the European Science Foundation, network Programme on Immunogenetics of Allergy (ESF) and by the SEAIC (Sociedad Española de Alergia e Inmunología Clínica).

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