

Segregation Analysis of the Specific Response to Allergens: A Recessive Major Gene Controls the Specific IgE Response to Timothy Grass Pollen

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Segregation analysis of the specific response to allergens (SRA) was performed in a sample of 234 randomly selected Australian families using the regressive models. Various SRA phenotypes were considered using broad and narrow definitions of these phenotypes, according to the type of test used, skin test or RAST test, and the specificity of the response to allergen. Strong evidence for familial dependencies among blood relatives was shown for most SRA phenotypes, especially when using a broad definition. There was no evidence for a Mendelian factor accounting for the familial transmission of these broadest phenotypes, which may involve multiple factors preventing the clear detection of a major effect with Mendelian transmission. However, segregation of a Mendelian recessive major gene was detected for one SRA sub-phenotype, the IgE response to a single allergen, Timothy grass pollen, measured by the RAST test. Identification of a specific SRA phenotype controlled by a major gene may have important implications for further linkage studies. *Genet. Epidemiol.* 16:305–315, 1999. © 1999 Wiley-Liss, Inc.

Key words: segregation analysis; specific response to allergens; RAST test; skin test; Timothy grass pollen

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INTRODUCTION

Allergy is defined as the propensity to raise immunoglobulin E (IgE) against environmental allergens. The presence of a genetic component in allergy is now well established but its exact nature is still unclear. To better understand the genetic determinism of allergy, segregation analyses focused mainly on total IgE levels. However, these analyses led to discordant results with some of them concluding to a recessive major gene [Marsh et al., 1974; Gerrard et al., 1978.; Meyers et al., 1987], other to a codominant one [Meyers et al., 1982] or to polygenic inheritance only [Hasstedt et al., 1983], and finally genetic heterogeneity was also suggested [Blumenthal et al., 1981]. Different modes of ascertainment of the data at different times of the year may partly explain these different results. The inheritance of total IgE levels may also result from a more complex mechanism than a single major gene effect. Separate genetic mechanisms may be involved in the basal IgE response and in the specific IgE response to allergens. A segregation analysis of total IgE levels taking into account the specific response to allergens (SRA) in a sample of 234 Australian families showed evidence for a recessive major gene effect involved in IgE regulation, acting independently of the SRA status [Dizier et al., 1995]. Further linkage analysis of this sample suggested linkage of this gene to the interleukin 4 (IL-4) cluster on chromosome 5q 31-33 [Dizier et al., 1998]. IgE levels were previously found to be linked to the IL-4 region in 11 Amish pedigrees [Marsh et al., 1994], with a higher evidence for linkage in a subset of sib-pairs with no detectable specific IgE response. These results in the Australian and Amish data suggest that an IL4-linked gene may be involved in basal IgE regulation. Analysis of 55 asthmatic Dutch pedigrees showed also significant linkage of chromosome 5q markers located 10 cM apart from the IL4-gene, to both IgE levels and bronchial responsiveness [Meyers et al., 1994; Postma et al., 1995]. Moreover, IgE levels and asthma have been recently reported to be linked to chromosome 12q in two different ethnic groups [Barnes et al., 1996]. All these results point out the complexity of the mechanisms involved.

With respect to the specific response to allergens, to our knowledge, no segregation analysis has yet been conducted. Association and non-parametric linkage studies have indicated the role of several candidate genes in SRA. HLA antigens were found associated with the specific IgE response to particular allergens [Freidhoff et al., 1988; Marsh and Huang, 1991]. Skin test responses to specific allergens were shown to be linked to the TCR- α gene on chromosome 14q in Australian and British families [Moffatt et al., 1994]. Moreover, the beta unit of the high affinity receptor to IgE might play a role in atopy defined by high IgE levels and/or positive skin tests [Cookson et al., 1989; Shirakawa et al., 1994]. New regions have also been suggested to be involved in asthma and allergy by two genome-wide searches [Daniels et al., 1996; CSGA, 1997].

The goal of the present study is to perform segregation analyses of SRA in the 234 Australian families, using various definitions of this trait, to better understand the different genetic mechanisms that may underlie the specific response to allergens. Detection of a major gene effect by segregation analysis may then increase the power to detect linkage by parametric methods using the characteristics of this gene.

DATA

The family data included 1,020 subjects belonging to 234 nuclear families from the West Australian Country town of Busselton. Families were recruited randomly through adults from an electoral roll of ~9,000 subjects. Families were admitted to the study if both parents were alive and under 55 years of age and if at least two children were older than 5 years. Mean age in parents is 40.21 ± 9.9 and in children 12.64 ± 9.4 . Both sexes are equally represented in this sample. The mean number of children per family is 2.35.

The information gathered on each family member included:

1. Total serum IgE (Phadezym PRIST, Pharmacia Ltd., Uppsala, Sweden)
2. Prick skin tests carried out to house dust mite (HDM), mixed grasses (Dhome-Holister-Steir, Spokane, WA), and mixed moulds with 50% glycerol as negative control. The weal diameter of the negative control was subtracted from the weal response to allergen.
3. Specific IgE titres to whole *Dermatophagoides pteronyssinus* (HDM) and *Phleum pratense* (Timothy grass) pollen extract were measured by ELISA (Phadezym CAP system, Pharmacia Ltd., Gaithersburg, MD)
4. Epidemiological covariates including sex, age, and smoking habits (past or present).

All clinical testing took place over 3 months in winter to control pollen exposure.

PHENOTYPES ANALYZED

Specific response to allergen is a complex phenotype that can be defined in different ways according to the type of test used, skin test or RAST test, the specificity of the response to a given allergen, the type of trait analyzed, binary or quantitative, and for binary traits the threshold used to define the positivity of the response. Different genetic mechanisms may be expected to control different SRA phenotypes. The following phenotypes were considered: (1) SRA: a positive response to at least one allergen either by RAST test or skin test; (2) RAST: a positive response to at least one allergen by RAST test; (3) SKT: a positive response to at least one allergen by skin test; (4) positive responses to house dust mite (HDM) by RAST test, RAST-HDM, and by skin test, SKT-HDM; (5) positive response to timothy grass (TG) by RAST test, RAST-TG, and to mixed grasses (MG) by skin test, SKT-MG; (6) positive response to mixed moulds (MM) by skin test, SKT-MM. The criterion used to define a positive response is a raised specific IgE level greater than 1 RAST units for the RAST test and a difference of weal with a negative control of at least 2 mm for the skin test, as used in prior studies [Dizier et al., 1995; Cookson et al., 1989]. We also considered more extreme thresholds for the response to skin test (1 and 5 mm) to assess how different thresholds may affect the conclusions of segregation analysis (SKT' and SKT'', SKT-HDM' and SKT-HDM'', SKT-MG' and SKT-MG'', SKT-MM' and SKT-MM'', for thresholds equal to 1 and 5 mm, respectively).

The quantitative measures of the RAST and skin tests were not analyzed by segregation analyses, since the distribution of these traits was highly skewed, even after transformation of the data.

The proportion of positive responses for each SRA phenotype and the two by two correlations (product moment correlation coefficient) between SRA phenotypes were computed using the BMDP package.

ASSOCIATION BETWEEN SRA AND COVARIATES

Association of SRA with covariates including age, sex, and generation were tested by logistic regression analysis using the BMDP package. Smoking habit was not considered since there were too many missing data.

SEGREGATION ANALYSIS

Segregation analyses of the different traits were conducted using the class D logistic regressive model [Bonney, 1986]. These models specify a regression relationship between each individual's phenotype and a set of explanatory variables including the effect of a di-allelic (a/A) major gene, a being the allele conferring a higher susceptibility to the disease (or here a positive response), the antecedents' phenotypes (to account for residual familial dependence of any origin: genetic and/or environmental), and measured covariates. The class D model assumes that, given parental outcomes and genotypes, the outcomes of siblings are equally predictive. For a given family, the likelihood can be written as: $L = \sum_g P_g \times P(Y/g, X)$, where Y is the vector of observed phenotypes of n individuals in the family, g the unobserved genotypes at the major locus and X the vector of measured covariates. P_g is the joint probability of the vector of genotypes and $P(Y/g, X)$ is the penetrance function. For individuals with no parent in the pedigree, P_g is a function of the allelic frequency, q_a , assuming Hardy-Weinberg equilibrium. For individuals with ancestors in the pedigree, P_g depends on the three transmission probabilities defined by Elston and Stewart [1971] for each parental mating type. These transmission parameters, denoted τ_{aaa} , τ_{Aaa} , and τ_{AAa} , are the conditional probabilities of transmitting to offspring allele a for parental genotypes aa, Aa, and AA, respectively. They are equal to 1, 0.5, and 0 under the Mendelian hypothesis, whereas they can take any value between 0 and 1 under the general transmission model.

The penetrance function $P(Y/g, X)$ is a multivariate logistic function decomposed in a product of univariate logistic functions by regressing each person's phenotype on the phenotypes of preceding relatives. Thus, the logit for the *i*th person is

$$\theta_i = \alpha_{gi} + \sum_A \gamma_A Y_A + \sum_k b_{gik} X_{ik}$$

and the probability to be affected is: $P(Y = 1/g_i, X_i, Y_A) = \exp(\theta_i) / (1 + \exp(\theta_i))$, where α_{gi} is the genotype-specific baseline risk, γ_A the regression coefficients on antecedents' phenotypes, Y_A ($A = \text{spouse, father, mother, and previous sib}$), b_{gk} the genotype-specific regression coefficients on covariate k. The antecedents' phenotypes Y_A were coded as 1 if the antecedent was affected and 0 if he/she was unaffected. This simple dichotomous coding scheme was used rather than a more general one [Demenais, 1991] since there were few persons with unknown phenotypes.

The parameters of the class D model are q_a , the allelic frequency of the major gene; α_{aa} , α_{Aa} , α_{AA} , the genotype-specific baseline parameters; τ 's, the transmission

probabilities; γ_s , γ_f , γ_m , γ_c the residual family dependencies between spouses, father-offspring, mother-offspring, and sib-sib, respectively; b_g , the genotype-specific regression coefficient for covariates.

The likelihood of the sample was computed using the computer program REGRESS [Demanais and Lathrop, 1994], a PASCAL program that incorporates the logistic regressive approach in the ILINK program of the LINKAGE package [Lathrop et al., 1984]. Different hypotheses are considered and tested by the likelihood ratio test. Evidence for familial aggregation is first asserted by testing a model with no familial dependence and no major gene effect against a model including familial dependencies only. Various patterns of familial dependence can then be tested. Evidence for the presence of a major gene is provided by testing a model with familial dependencies and no major effect against a model including both types of effects. If segregation of a major effect is detected, interactions between this major gene effect and the measured covariates are searched. To support the presence of this major gene, two additional criteria on transmission probabilities have to be fulfilled: non-rejection of the Mendelian transmission hypothesis of this major effect (τ_{aaa} , τ_{Aaa} , and τ_{AAa} being, respectively, equal to 1, 0.5, 0) and rejection of the absence of parent-offspring transmission (equal τ 's) against the general transmission probability model (where the three τ 's can take any value between 0 and 1).

RESULTS

Association Between SRA Phenotypes and Covariates

All SRA phenotypes considered were found associated significantly with age, age² and sex, but there was no generation effect. No interactive effect among these covariates was detected. The significant covariates were then included in segregation analyses.

Distribution of SRA Phenotypes in Our Family Sample

As seen in Table I, the proportion of individuals with a positive response to at least one allergen is similar using either the RAST test (RAST: 52%) or the skin test (SKT: 55%, with a threshold of 2 mm), and only slightly higher when pooling both tests (SRA: 61%). When considering other phenotypes, the proportion of subjects with a positive response to HDM or grasses is slightly decreased, about 40%, as compared to the global response, and in a larger extent for mixed moulds (17%). The proportions of positive responses are still similar using the RAST or skin test to HDM and grasses. Moreover, the distributions of the skin test responses are similar with the thresholds of 1 and 2 mm whereas the proportions of positive responses are greatly decreased when using the threshold of 5 mm.

The two by two correlations between SRA phenotypes are shown in Table II. They are all highly significant (P values <0.001). The highest correlations (>0.8) are observed between phenotypes (SRA, SKT, RAST) defined by a positive response to at least one allergen and differing for the type of test used. Similarly, correlations between response to a specific allergen but using different tests (e.g., RAST-HDM and SKT-HDM with a correlation of 0.72) are higher than those between responses to different allergens and using the same test (e.g., RAST-HDM and RAST-TG with a correlation of 0.44). The smallest observed correla-

TABLE I. Proportions of Subjects With a Positive Response by SRA Phenotypes

Type of allergen	Type of test				
	RAST and/or skin test	RAST test	Skin test		
At least one allergen	SRA 61%	RAST 52%	SKT 55%	SKT' 54%	SKT'' 39%
House dust mite		RAST-HDM 37%	SKT-HDM 48%	SKT-HDM' 47%	SKT-HDM'' 25%
Timothy grass		RAST-TG 41%			
Mixed grasses			SKT-MG 36%	SKT-MG' 35%	SKT-MG'' 17%
Mixed moulds			SKT-MM 17%	SKT-MM' 14%	SKT-MM'' 2%

tions were between the response to mixed moulds by skin test (SKT-MM) and any other SRA phenotypes.

Segregation Analysis of SRA Phenotypes

Tests of familial dependencies

Segregation analysis first assessed the patterns of family dependencies among relatives without including a major gene effect. For each SRA phenotype, Table III presents the familial dependence parameters and regression coefficients for covariates as well as the likelihood-ratio tests. Each type of familial dependency (spouse, father-offspring, mother-offspring, sib-sib) was alternatively tested by comparing a model without the tested dependency to the general model including all types of dependencies. Absence of familial dependencies among blood relatives (father-offspring, mother-offspring, and sib-sib) were also globally tested by comparing a model without these dependencies to the general model (including all of them). The spouse dependency was significant for only three phenotypes, SRA, SKT, and SKT-MG ($\chi^2_{1df} = 4.2, 5.3$ and 5.2 , $P = 0.04, 0.02$, and 0.02 , respectively). There is a strong evidence for familial dependence among blood relatives for all phenotypes, with the smallest effects obtained with the SKT-MM ($\chi^2_{3df} = 16.3$, $P = 0.001$) and the highest ones with the broadest SRA phenotype ($\chi^2_{3df} = 59.9$, $P < 10^{-6}$). The SKT phenotype displays a higher degree of familial aggregation ($\chi^2_{3df} = 49.5$, $P < 10^{-6}$) than RAST

TABLE II. Two by Two Correlation Coefficients Between SRA Phenotypes*

	SRA	RAST	RAST-HDM	RAST-TG	SKT	SKT-HDM	SKT-MG	SKT-MM
SRA	1.	0.83	0.61	0.66	0.87	0.75	0.58	0.33
RAST		1.	0.73	0.79	0.69	0.69	0.54	0.27
RAST-HDM			1.	0.44	0.66	0.72	0.43	0.26
RAST-TG				1.	0.50	0.48	0.62	0.29
SKT					1.	0.87	0.66	0.38
SKT-HDM						1.	0.53	0.33
SKT-MG							1.	0.35
SKT-MM								1.

*Product moment correlation coefficient.

TABLE III. Test of Familial Dependencies for each SRA Phenotype*

Phenotypes	Parameters								Test of no dependency between blood relatives (χ^2 , 3df) ^a
	α	γ_s	γ_f	γ_m	γ_c	bsex	bage	bage ²	
SRA	-1.27	0.49	0.65	0.79	0.27	-0.52	0.12	-0.002	59.9
		(4.5)	(12.8)	(17.5)	(4.0)				
RAST	-1.09	0.36	0.72	0.64	0.33	-0.55	0.09	-0.001	37.2
		(2.3)	(15.7)	(12.5)	(5.8)				
RAST-HDM	-1.97	0.17	1.11	0.57	0.45	-0.38	0.11	-0.002	54.2
		(0.3)	(34.0)	(7.9)	(8.3)				
RAST-TG	-0.9	0.27	0.59	0.58	0.41	-0.50	0.04	-0.001	32.7
		(1.2)	(1.04)	(9.5)	(8.3)				
SKT	-1.98	0.53	0.85	0.74	0.27	-0.42	0.15	-0.002	49.5
		(5.3)	(22.2)	(16.0)	(3.7)				
SKT-HDM	-2.3	0.18	0.86	0.83	0.26	-0.45	0.16	-0.003	48.4
		(0.8)	(23.0)	(20.2)	(3.3)				
SKT-MG	-1.89	0.62	0.74	0.53	0.44	-0.22	0.09	-0.001	36.9
		(5.2)	(14.3)	(7.6)	(7.9)				
SKT-MM	-2.91	0.62	1.01	0.56	0.17	0.06	0.10	-0.002	16.3
		(1.5)	(9.5)	(3.0)	(0.4)				

*In parentheses are given the χ^2 values corresponding to the test of absence of each familial dependency.

^aThe test is conducted by comparing a model including spouse dependency alone vs. a model including all familial dependencies (between spouses, father-offspring, mother-offspring, sibling).

($\chi^2_{3df} = 37.2$, $P < 10^{-6}$). The specific response to the house dust mite shows stronger evidence for familial dependencies ($\chi^2_{3df} = 54.2$ and 48.4 , $P < 10^{-6}$, for RAST-HDM and SKT-HDM, respectively) than the response to grasses ($\chi^2_{3df} = 32.7$ and 36.9 , $P < 10^{-6}$, for RAST-TG and SKT-MG, respectively). For all phenotypes, father-offspring dependency does not differ significantly from mother-offspring dependency, except for RAST-HDM ($\chi^2_{1df} = 3.9$, $P = 0.05$). Evidence for sib-sib dependencies is smaller than for parent-offspring dependencies for most phenotypes, except for RAST-TG and SKT-MG.

When changing the threshold for the skin test response, familial dependencies are similar when using the threshold of 1 mm instead of 2 mm but are weaker with the threshold of 5 mm (results not shown).

Tests of a major gene effect

When comparing a model with familial dependencies and no major gene effect against a model including these two components, no major gene effect was detected for the SRA, RAST, and SKT phenotypes ($\chi^2_{3df} = 4.8$, $P = 0.19$, $\chi^2_{3df} = 4.9$, $P = 0.18$, $\chi^2_{3df} = 6.2$, $P = 0.10$, respectively), as well as for the response to house dust mite (RAST-HDM, $\chi^2_{3df} = 4.6$, $P = 0.20$ and SKT-HDM, $\chi^2_{3df} = 5.3$, $P = 0.15$) and the response to mixed moulds SKT-MM ($\chi^2_{3df} = 0.8$, $P = 0.85$). However, there is evidence for a major factor accounting for the familial transmission of the response to grasses, either the specific response to Timothy grass using the RAST test (RAST-TG, $\chi^2_{3df} = 9.1$, $P = 0.03$) or the response to mixed grasses using the skin test (SKT-MG, $\chi^2_{3df} = 12.3$, $P = 0.006$). Results of segregation analyses of these two phenotypes are presented in Tables IV and V. Note that for these latter phenotypes (RAST-TG and SKT-MG), the evidence for familial dependencies in the absence of a major

TABLE IV. Segregation Analysis of RAST Test to Timothy Grass Pollen (RAST-TG)*

q_a	α_{aa}	α_{Aa}	α_{AA}	γ_s	γ_f	γ_m	γ_c	b_{sex}	b_{age}	b_{age2}	τ_{aaa}	τ_{aAa}	τ_{AAa}	$-2\ln(L)$
No Major Gene, familial dependencies														
—	-0.9	[-0.9]	[-0.9]	0.27	0.59	0.58	0.41	-0.5	0.04	-0.001	[1]	[0.5]	[0]	1295.8
Recessive Major Gene, familial dependencies														
0.75	1.44	-2.2	[-2.2]	0.38	0	0	0	-0.8	0.02	-0.001	[1]	[0.5]	[0]	1286.7
Dominant Major Gene, familial dependencies														
0.56	-0.3	[-0.3]	-8.11	0.33	0.53	0.52	0.27	-0.6	0.04	-0.001	[1]	[0.5]	[0]	1293.6
Recessive Major Factor without parent-offspring transmission, familial dependencies														
0.53	-0.63	-1.52	[-1.5]	0.31	0.62	0.59	0.44	-0.51	0.05	-0.001	0.26	0.26	0.26	1295.4
Recessive Major factor, general parent-offspring transmission, familial dependencies														
0.60	0.97	-4.1	[-4.1]	0.8	0	0	0	-1.33	0.12	-0.002	0.9	0.21	0	1281.5

*Values in brackets represents fixed parameters.

gene effect was weaker than for the phenotypes where no major effect was detected. For RAST-TG, dominant transmission of the major factor is rejected ($\chi^2_{1df} = 6.9$, $P = 0.009$), while the recessive one is not ($\chi^2_{1df} = 0$). Under the recessive model, residual familial dependencies among relatives converge to zero ($\chi^2_{3df} = 0$) and the spouse dependency is not significantly different from zero. There is no significant interaction between the major effect and the covariates (results not shown). Tests of transmission probabilities allow the confirmation of the presence of a major gene since the transmission of this major factor fits Mendelian transmission ($\chi^2_{3df} = 5.2$, $P = 0.16$) and the absence of parent-offspring transmission is rejected ($\chi^2_{2df} = 13.9$, $P = 0.001$). The allelic frequency of this recessive major gene is equal to 0.75 and the genotype-dependent risks in males and in females for different ages are given in Table VI.

Results obtained for SKT-MG are quite different. Neither a dominant nor a recessive mode of transmission of the detected major factor is rejected. No interaction is observed between the major effect and the covariates. Transmission of a major gene cannot be asserted since absence of parent-offspring transmission is not rejected ($\chi^2_{2df} = 0.9$, $P = 0.64$).

Using more extreme thresholds for the skin tests (1 or 5 mm), no evidence for a

TABLE V. Segregation Analysis of Skin Test to Mixed Grass Pollen (SKT-MG)*

q_a	α_{aa}	α_{Aa}	α_{AA}	γ_s	γ_f	γ_m	γ_c	b_{sex}	b_{age}	b_{age2}	τ_{aaa}	τ_{aAa}	τ_{AAa}	$-2\ln(L)$
No Major Gene, familial dependencies														
—	-1.89	—	—	0.62	0.74	0.53	0.44	-0.22	0.09	-0.001	[1]	[0.5]	[0]	1241.2
Major Gene, familial dependencies														
0.32	-1.6	-0.4	-12.1	2.1	0.73	0	0	-0.53	0.14	-0.003	[1]	[0.5]	[0]	1228.1
Recessive Major Gene, familial dependencies														
0.76	-0.34	-11	[-11]	0.91	0.08	0	0	-0.41	0.10	-0.002	[1]	[0.5]	[0]	1229.7
Dominant Major Gene, familial dependencies														
0.33	-0.5	[-0.5]	-11.8	1.6	0.4	0	0.004	-0.48	0.12	-0.002	[1]	[0.5]	[0]	1228.9
Dominant Major factor without parent-offspring transmission, familial dependencies														
0.24	-2.9	[-2.9]	-19.7	11.0	6.5	0.97	2.26	-0.44	0.28	-0.004	0.71	0.71	0.71	1227.9
Dominant Major factor, general parent-offspring transmission, familial dependencies														
0.70	-11.1	-0.9	[-0.9]	2.8	0.23	0	0	-0.44	0.14	-0.002	0.94	0.15	1.0	1225.7

*Values in brackets represents fixed parameters.

TABLE VI. Genotype-Dependent Risk for a Positive Response to Timothy Grass Using the RAST Test (RAST-TG)

	Sex					
	Male			Female		
Age (years)	10	20	40	10	20	40
Genotypes						
aa	0.83	0.78	0.72	0.70	0.63	0.56
aA (=AA)	0.13	0.10	0.07	0.06	0.05	0.04

major effect is obtained for the following phenotypes: SKT', SKT', SKT-HDM', SKT-HDM', SKT-MM' and SKT-MM' as when using a 2-mm threshold. However, analysis of the positive response to mixed grasses defined with a threshold of 1 mm (SKT-MG') leads to the detection of a major effect, which is not confirmed by the tests of transmission probabilities, similarly to the results obtained with the 2-mm threshold. However, when using the threshold of 5 mm, there is no longer evidence for a major factor. These results point out the influence of the threshold values on the outcomes of segregation analysis.

DISCUSSION

The complexity of the genetic mechanisms underlying the specific response to allergens could be anticipated given the high number of allergen sources involved, each giving rise to several major antigens that contain each approximately three common epitopes (antigen recognition sites). To better understand the complex determinism of SRA, we considered different SRA phenotypes, which were likely to involve different genetic mechanisms. Altogether, our results demonstrate the influence of the definition of the phenotype on the outcomes of segregation analyses. A major gene effect is only detected for one of the 13 phenotypes studied, the RAST test response to Timothy grass, although all these phenotypes were strongly correlated. The gene shown to increase the risk of a positive response is recessive with an allelic frequency equal to 0.75. Although a major effect was also detected for the skin test response to mixed grasses, the non-rejection of the parent-offspring transmission of this factor prevented to confirm the presence of a major gene. The reason of some similarity between these skin test and RAST results may be that there is strong cross-reactivity between the major allergens from various grass species. Note that the correlation of RAST-MG was higher with SKT-MG (0.66) than with SRA phenotypes defined by the response to other allergens (RAST-HDM, SKT-HDM, SKT-MM). However, the skin test response appears to be a more complex trait than RAST and effects of multiple factors on this trait may alter the detection of a major factor transmitted in a Mendelian manner. The RAST test measures the response to a single allergen, Timothy grass, while the skin test measures the response to mixed grasses which contain several grass pollens. Moreover, the response to skin test involves more biological pathways than RAST (i.e., binding of IgE to allergens, fixation of the complex IgE-allergen on mastocytes, degranulation of mastocytes).

The positive response to at least one allergen and the response to house dust mite show stronger familial dependencies than the response to grasses, although

no major gene effect was detected for these phenotypes. Again, these phenotypes are likely to result from multiple factors, genetic and/or due to shared environment, which may prevent the clear detection of a major gene. These results point out the need to consider sub-entities of a complex phenotype, where Mendelian factors can be evidenced.

Identification of a recessive Mendelian factor involved in one SRA sub-phenotype, the RAST test to Timothy grass, without residual familial dependencies, may have important implications for further linkage studies by delineating the phenotype to be analyzed and the characteristics of the gene. Thus, a more stringent definition of the phenotype may not only help in revealing a major gene effect by segregation analysis but also may increase the power of detecting linkage.

As mentioned in the Introduction, several candidate regions have been reported to be associated and/or linked to different phenotypes associated with allergy, including 5q [Marsh et al., 1994; Meyers et al., 1994; Postma et al., 1995] and 12q [Barnes et al., 1996] regions for serum IgE levels and HRB, 11q for atopy [Cookson et al., 1989; Shirakawa et al., 1994] and 14p [Moffatt et al., 1994] for specific IgE response. Associations have been found between HLA antigens and different allergens [Freidhoff et al., 1988; Marsh and Huang, 1991]. The TCR- α gene on chromosome 14q and HLA are the most likely candidate regions involved in SRA (Moffatt and Cookson, 1997). The molecules encoded by HLA and TCR genes are central to the handling and recognition of exogenous antigens. However, association and/or linkage with these regions were found with different allergens including the mixed grasses but also house dust mite and other allergens. It would be of interest to search whether the recessive gene shown to control the positive response to Timothy grass by the RAST test in the Australian families is located in one of these two candidate regions or elsewhere.

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