

Skin Testing Correlates Negatively with High-Activity ACP1 *B/*C Genotype

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Key Words

Skin prick test • Genetic polymorphism • ACP1

Abstract

Background: Previous studies have shown a negative association between ACP1 *B/*C genotype and total IgE level. ACP1 (acid phosphatase locus 1) is a polymorphic phosphotyrosine phosphatase that interacts with IL4-RA and is involved in T cell receptor signaling. **Methods:** In the present paper, we have studied the relationship between *B/*C genotype which shows high ACP1 activity and skin testing in 300 adult subjects referred for allergic manifestations. ACP1 genotypes were determined by DNA analysis. **Results:** There is a significant negative correlation between the intensity of skin test reaction and *B/*C genotype ($p = 0.01$). The proportion of *B/*C genotype is lower in allergic subjects with intense skin reaction than in allergic subjects with moderate skin reaction and in healthy controls. **Conclusions:** This new observation confirms by a different approach the relationship between ACP1 polymorphism and allergic manifestations, suggesting that high ACP1 activity protects against these manifestations.

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Introduction

ACP1 (acid phosphatase locus 1 or low molecular weight protein tyrosine phosphatase, LMPTP) is one of the few phosphotyrosine phosphatases with a known genetic polymorphism associated with strong quantitative variation of enzymatic activity. Previous observations suggest an involvement in allergic manifestations [1]. Recently, an association between ACP1 and total IgE levels has been reported in 2 populations: in ACP1 *B/*C genotype that shows a high enzymatic activity, the level of total IgE is lower than in other genotypes [2].

In the present paper we have investigated the possible association between *B/*C genotype and the reaction of skin to the most common allergens.

ACP1 Genetic Polymorphism

The polymorphism of ACP1 was discovered initially at protein level by Hopkinson et al. [3], who described 6 different patterns on starch gel electrophoresis of hemolysates. These authors attributed these patterns to the combination of 3 codominant alleles named *A, *B and *C at an autosomal locus. At present, the term ACP1 is used to indicate the gene, while the protein is indicated by the term LMPTP.

The enzyme resolved by nondenaturing starch gel electrophoresis into 2 isoforms called fast (F) and slow (S). The amino acid sequence of LMPTP [4, 5] revealed that the 2 isoforms are encoded by a single gene and arise through a pre-mRNA splicing process, in which either exon 3 or 4 is excised and the other retained [6, 7].

The 3 ACP1 alleles have been sequenced [8] and found to be based on 3 single nucleotide polymorphisms (SNPs) that affect both the total enzymatic activity and the ratio [9] between the F and S isoforms in a characteristic manner, thus explaining the different pattern seen in starch gel electrophoresis. Fixed combinations of these SNPs define 6 common genotypes **A/*A*, **B/*B*, **C/*C*, **B/*A*, **C/*A* and **C/*B*. In the **A* allele, the amino acid 105 (exon 6) is an arginine residue (codon CGA), while in the **B* and **C* alleles it is a glutamine (codon CAA). The other 2 SNPs (codon 41 at exon 3 and codon 43 at exon 4) do not change the encoded amino acid residues but strongly affect the alternative mRNA splicing and the ratio between the 2 isoforms. As a result, the F/S ratio is 2:1 in **A*, 4:1 in **B* and 1:4 in **C*.

ACP1 is present at high concentration in many tissues [10]. The total enzymatic activity, measured with *p*-nitrophenylphosphate as a substrate, is in the order **A/*A* < **A/*B* < (**B/*B*, **A/*C*) < **B/*C* < **C/*C*.

ACP1 and IL-4

Among the immune signal transduction pathways affecting IgE production, the IL-4 receptor signal transduction is one of the more important [11]. Some transduction pathways activated by the IL-4 receptor stimulation are similar to pathways activated during insulin signal transduction, which is in vitro modulated by the S isoform of ACP1 [12]. Phosphorylated Tyr residues, preceded by a couple of non-bulky hydrophobic residues, could represent the preferential target for LMPTP-S dephosphorylating action [13]: IL-4 receptor α -chain (IL-4RA) is phosphorylated on 5 Tyr residues after stimulation by IL-4.

A significant interaction between ACP1 polymorphism and intracellular Gln/Arg polymorphism in portion 551 of IL-4RA in relation to the predisposition to high total IgE levels has been described [14], suggesting a direct or indirect biochemical interaction between ACP1 and IL-4RA, and resulting in different modulation of IL-4 signal transduction among joint genotypes.

ACP1 and T Cell Receptor Signaling

ACP1 specifically dephosphorylates the negative regulatory Tyr-292 of ZAP-70, counteracting inactivation of

Table 1. Clinical and demographic data of the sample studied

Male, %	49.3
Age at diagnosis, years	25.8 $\pm$ 0.9
Asthmatic, %	38.3
Allergens	Positive subjects
Dermatofagoides	158
Grass	133
Parietaria officinalis	118
Olive	69
Cupressus	61
Alimentary	16
Cat	28
Dog	8
Other	19

ZAP-70. The ZAP-70 is a protein-tyrosine kinase that plays a central role in signaling from the T cell antigen receptor. Recruitment and activation of ZAP-70 are terminated by phosphorylation of negative regulatory tyrosine residues and dephosphorylation of positively acting sites. Thus, ACP1 dephosphorylating a negative regulatory tyrosine site in a protein-tyrosine kinase strengthens T cell receptor signaling [15].

Material and Methods

Subjects

Three hundred subjects presenting for allergic manifestations including asthma, rhinitis and conjunctivitis have been studied in the Caucasian population of Rome. Clinical and demographic data are reported in table 1. All subjects gave informed verbal consent to participate in the present study that was approved by the Institutional Review Board.

Eight hundred and four healthy subjects from the same population previously studied by us were considered as controls.

ACP1 Genotype Determination

The difference between the ACP1 **A* and **B* alleles at codon 105 exon 6 (A-G transition) and the difference of the **C* allele from the **A* and **B* alleles at codon 43 exon 3 (C-T transition) were utilized for ACP1 genotype determination.

Total genomic DNA was extracted from frozen whole-blood samples collected in NA-EDTA using the procedure described by Kunkel et al. [16] with slight modifications. All polymerase chain reactions (PCRs) were set up in 30 μ l buffer containing 0.2 μ mol/l of both primers, 0.1 mmol/l dNTPs, 1.5 mmol/l MgCl₂, 0.5 U of Taq polymerase (AmpliTaQ; Applied Biosystems, Mannheim, Germany), 1 $\times$ AmpliTaq buffer (phycoerythrin) and 50 ng of DNA template. The amplification conditions consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles

Table 2. Primers used for ACP1 SNP analysis

Primer No.	Target amplification	Nucleotide sequence
263	exon 3 forward	GTCGATCACCCATTGCAGAAGC
264	exon 3 reverse	CACACAACCTTCAAGTCCAAGGACG
267	exon 6 forward	GGATGTTTTCAGAAGACCCTAGCAG
268	exon 6 reverse	GCTCCCAAGTAGTTCAATTTTAGC

Table 3. Distribution of ACP1 genotypes in allergic subjects and controls from the same Caucasian population

	ACP1 genotypes, %						Total n
	*A/*A	*B/*B	*C/*C	*A/*B	*A/*C	*B/*C	
Allergic subjects	8.0	47.3	0.3	31.7	5.3	7.3	300
Controls	9.6	42.5	0.1	33.0	3.6	11.2	804

The difference between the 2 groups was significant for the *B/*C genotype: $p = 0.034$, χ^2 test of independence (under the hypothesis of protective effect).

at 94°C for 45 s, 54°C for 45 s, 72°C for 45 s and a final extension at 72°C for 5 min. The annealing temperature, extension time and primer concentration for the 2-kb amplification product were 57°C, 120 s and 0.1 $\mu\text{mol/l}$, respectively.

Oligonucleotide primers used for PCR amplification are reported in table 2. The C-T transition at codon 43 and the A-G transition at codon 105 generate a Cfo and Taq 1 restriction site that, together, were used for PCR-based genotyping (modified from Lazaruk [17]). A 341-bp segment spanning the entire exons 3 and 4 was amplified using primers No. 263 and 264 (table 2). A 299-bp segment including exon 6 was amplified using primers No. 267 and 268. Ten microliters of the 341-bp exon 3 amplicon was fully cleaved by Cfo1 at 37°C for 1 h according to the manufacturer's instructions and then electrophoresed on 1.8% agarose gels. The digestion created 2 fragments of 255 and 86 bp for the ACP1 *A and *B alleles, while the *C allele was not cut. Similarly, the 299-bp PCR product was digested by Taq 1 at 65°C for 1 h according to the manufacturer's instructions, generating 2 fragments of 100 and 199 bp for the ACP1 *A allele, but not for the *B and *C alleles (modified from Lazaruk [17]).

Skin Prick Test

Skin prick test was performed in all subjects with a panel of the local allergens (Lofarma Allergeni SPA, Milan): *Dermatophagoides*, *Poa pratensis* (grass), *Parietaria officinalis*, *Olea Europea* (olive), *Cupressus*, milk, albumin, egg white, cat and dog hair.

Histamine chloride (1 mg/ml) and control solution were also tested at the distal end of the forearm. Allergens were applied to the volar side of both forearms and tested in the order above with a distance of about 2 cm between each application. Ten to fifteen minutes after skin prick, the mean allergen wheal diameter (minus the diameter of the control solution wheal, when this differed from zero) was recorded. Each allergen test was also evaluated, comparing its wheal with the size of the histamine wheal (1 mg/ml): allergen wheals half the size of the positive reference or bigger were considered positive.

A semiquantitative evaluation of intensity of a positive reaction was obtained comparing its wheal with the size of the histamine wheal: size equal or smaller than that of histamine was classified as moderate, size greater than that of histamine was classified as intense.

Statistical Methods

χ^2 test of independence was carried out according to SPSS (version 5; SPSS Inc., Chicago, Ill., 1992) using an IBM PC. Cumulative probability was calculated according to Sokal and Rohlf [18].

Results

Table 3 shows the distribution of ACP1 genotypes in allergic subjects and controls. In allergic subjects, ACP1 genotype distribution does not show significant difference from Hardy-Weinberg expectation ($p = 0.12$). The proportion of *B/*C genotype in allergic subjects is lower than in controls ($p = 0.034$).

Table 4 shows the proportion of allergic subjects carrying the *B/*C genotype in relation to the intensity of cutaneous skin prick test reaction. For all allergens, a negative correlation is found between the intensity of reaction and the presence of *B/*C genotype. The proportion of *B/*C genotype in allergic subjects with intense reaction is lower compared to both controls and allergic subjects with moderate skin reaction. An overall odds ratio of 0.33 (95% confidence interval 0.151–0.741) points to a protective action of the *B/*C genotype against intense skin reaction.

Table 4. Proportion of subjects with ACP1 *B/*C genotype in relation to intensity of skin prick test reaction

Allergens	Patients (skin prick test reaction)				Controls		Significance of difference		
	moderate		intense				between moderate and intense	between patients and controls	
	*B/*C, %	total, n	*B/*C, %	total, n	*B/*C, %	total, n		versus moderate	versus intense
<i>Dermatofagoides</i>	14.3	49	5.5	109	11.2	804	p = 0.07	p = 0.50	p = 0.07
Grass	15.1	33	6.0	100	11.2	804	p = 0.10	p = 0.50	p = 0.12
<i>P. officinalis</i>	15.8	19	5.3	99	11.2	804	p = 0.10	p = 0.65	p = 0.07
Cumulative probability							p = 0.025	p = 0.70	p = 0.02

Table 5. The relationship between *B/*C genotype and cutaneous prick test reaction to 2 allergens

Allergens	Degree of skin reaction						Significance of difference between degree of skin reaction	Correlation between degree of skin reaction and proportion of *B/*C genotype
	moderate		moderate intense		intense			
	*B/*C	n	*B/*C	n	*B/*C	n		
<i>Dermatofagoides</i> and grass	27.3%	11	12.9%	31	2.4%	41	p = 0.034	p = 0.010
<i>Dermatofagoides</i> and <i>Parietaria</i>	33.3%	6	13.8%	29	3.1%	32	p = 0.063	p = 0.022
Grass and <i>Parietaria</i>	33.3%	6	12.5%	24	2.6%	39	p = 0.032	p = 0.012

Table 6. The relationship between *B/*C genotype and cutaneous prick test reaction to 3 allergens in females

Allergens	Degree of skin reaction								Significance of difference between degree of skin reaction	Correlation between degree of skin reaction and proportion of *B/*C genotype
	moderate		moderate		moderate		intense			
	moderate		moderate		intense		intense			
	moderate		intense		intense		intense			
	*B/*C	n	*B/*C	n	*B/*C	n	*B/*C	n		
<i>Dermatofagoides</i> , <i>Parietaria</i> and grass	100%	1	23.1%	13	7.7%	13	0%	18	p = 0.006 ^a p ≈ 0.032 ^b	p = 0.004

^a χ^2 test of independence. ^b Permutation test.

Table 5 shows the relationship between *B/*C genotype and cutaneous prick test reaction to 2 allergens. There is a negative correlation between the proportion of *B/*C subjects and degree of reaction to allergens considering both intensity and number of allergens tested.

Table 6 shows the relationship between *B/*C genotype and cutaneous prick test reaction to 3 allergens. There is a highly significant negative correlation between

the proportion of *B/*C subjects and degree of reaction considering both intensity and number of allergens tested.

These results suggest a protective action of *B/*C genotype towards intense cutaneous reactions against the allergens considered. The negative association between *B/*C genotype and skin prick test is more evident in males than in females, but the difference between gender is not statistically significant (data not shown).

Discussion

Based on previous observations showing a negative correlation between ACP1 *B/*C genotype and total IgE level, our working hypothesis was that a similar negative correlation could also exist between *B/*C genotype and skin prick test. All analyses of this paper show the presence of such a correlation, thus supporting our working hypothesis and pointing to a protective action of *B/*C genotype against allergic manifestations. From a statistical point of view, since previous studies have shown a negative correlation between *B/*C and IgE level, the a priori probability to find a similar association between *B/*C and skin test positivity is definitely different from zero. Thus, the test should be directional and the probability of type 1 error is sensibly lower than that shown in tables 4–6.

Many genes codifying for molecules involved in signal transduction processes have been proposed as candidates for atopy based on both the results of linkage analysis studies and on the knowledge of the pathogenic mechanisms of IgE hyperproduction. The IL-4/IL-13 signal transduction system has extensively been investigated in relation to the genetic predisposition to atopy and allergic diseases [19].

The level of phosphorylation on tyrosine of intracellular molecules in basal conditions and after stimulation depends on the balanced action of kinases responsible for phosphorylation processes, and of phosphatases which

are responsible for dephosphorylating previously phosphorylated residues.

Among the signal transduction pathways involved in IgE hyperproduction, the IL-4 receptor signal transduction may be a good candidate for the ACP1 regulatory role. Several tyrosine phosphorylation phenomena are activated following IL-4 stimulation, and many pathways activated by the IL-4 receptor are similar to those activated by insulin [11], whose signal transduction is modulated by the ACP1 S isoform.

Low ACP1 activity is seen to increase insulin signaling, whereas high ACP1 activity is able to depress it. Considering the similarities between insulin and IL-4 receptor signaling, one could envision that ACP1 has a similar effect on the 2 signaling systems. Thus, low ACP1 activity would strengthen the effects of IL-4, resulting in an increased susceptibility to allergic manifestations, while high ACP1 activity would elicit an opposite effect.

The role of ACP1 in T cell receptor signaling through modulation of ZAP-70 activity could also have a role in influencing Th1/Th2 balance. The dephosphorylation of an inhibitory phosphorylation site on the kinase ZAP-70 results in an increase in enzymatic activity of ZAP-70. As high ZAP-70 activity has been reported to favor the polarization of T helper cells toward a Th1 phenotype [20], a low ACP1 activity would rather skew T cell differentiation toward a Th2 phenotype, while high ACP1 activity would elicit an opposite effect.

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