

ACP1 and Th Class of Immunological Disease: Evidence of Interaction with Gender

F. Gloria-Bottini^a N. Bottini^b G. Renzetti^c E. Bottini^a

Departments of ^aBiopathology and Imaging Diagnostics and ^bInternal Medicine, University of Rome Tor Vergata, Rome, and ^cDepartment of Pediatrics, City General Hospital, Pescara, Italy

Key Words

ACP1 · Th class · Immunological diseases · Th1/Th2 orientation, gender

Abstract

Background: Data collected by our group in the past years indicate a relationship between ACP1 genetic polymorphism and susceptibility/resistance to immunological diseases. Recent observations suggest that through modulation of ZAP-70 activity, the enzyme may influence T cell activation. In view of the current interest in gender differences in autoimmune diseases we reviewed our data to enlighten possible effects of gender on the relationship between ACP1 and class of immunological disease. **Methods:** We studied three samples of subjects with allergic disorders of a total of 299 subjects, 71 subjects with Crohn's disease and 188 children with type 1 diabetes. Three-way contingency tables were analyzed by a log linear model and two-way contingency tables by χ^2 test. **Results:** There is an association between ACP1 and allergy (Th2 class) that depends on gender: the presence of the ACP1*A allele seems to make females more susceptible to allergic manifestations as compared to males. ACP1 is also associated with Crohn's disease and type 1 diabetes: the relationship between this class (Th1) of immunological diseases and ACP1 depends on gender. The presence of *A allele seems to make females less susceptible to this class of diseases as compared to males. **Conclu-**

sions: The ACP1*A allele which is associated with low ACP1 activity appears responsible for a complex relationship involving gender, ACP1 and Th1/Th2 orientation. Low ACP1 activity influencing ZAP-70 activity and in turn T cell activation seems to have opposite effects on Th1/Th2 orientation depending on gender.

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Introduction

ACP1 (acid phosphatase locus 1) or cLMWPTP (cytosolic low molecular weight protein tyrosine phosphatase) is a polymorphic enzyme showing strong quantitative variations of total enzymatic activity among genotypes. In Caucasian populations, six genotypes are present: *A/*A, *A/*B, *B/*B, *A/*C, *B/*C and *C/*C attributed to the presence of three codominant alleles, ACP1*A, ACP1*B and ACP1*C, at an autosomal locus. Spencer et al. [1] found the following activities ($\mu\text{mol } p\text{-nitrophenol produced in 30 min/g hemoglobin at } 37^\circ\text{C}$): ACP1*A/*A: 122.4, ACP1*A/*B: 153.9, ACP1*B/*B: 188.3, ACP1*A/*C: 183.8 and ACP1*B/*C: 212.3. The enzyme is composed of two isoforms, F and S, which have different molecular and catalytic properties [2, 3].

Each ACP1 allele produces two distinct isozymes generated by an alternative splicing mechanism [4]. The two isozymes called 'fast' (F) and 'slow' (S) according to their

Table 1. Mechanisms by which ACP1 may act at receptorial level

Carriers of *A allele	→	Low dephosphorylating activity on the negative regulatory Tyr 292 of ZAP-70	→	Low ZAP-70 activity	→	Weakening of T cell receptor signaling
Other ACP1 genotypes	→	High dephosphorylating activity on the negative regulatory Tyr 292 of ZAP-70	→	High ZAP-70 activity	→	Strengthening of T cell receptor signaling

anodal mobility show different molecular and catalytic properties. Both the total amount of enzyme produced and the relative proportion of the two isoforms are different among ACP1 alleles and phenotypes. Recent evidence suggests that the 5' flanking region of the ACP1 gene includes sequences operating in strict functional association with coding sequences probably in order to regulate the F/S ratio of the *A and *B alleles [5].

*A and *B alleles are present in all human populations: the *B allele, more common in all populations, shows an F/S ratio equal to 4.2. This ratio is reduced to 2.4 for the less common *A allele.

Two separate functions have been attributed to ACP1: phosphoprotein tyrosine phosphatase (PTP) and flavin mononucleotide phosphatase (FMNP).

Overexpressed LMPTP can counteract the malignant transformation by tyrosine kinase oncogenes [6] as well as signaling by growth factor receptors [7–10]. Our laboratory has found that LMPTP dephosphorylates a negative regulatory phosphorylation site in the ZAP-70 tyrosine kinase in T cells [11]. This event leads to increased activation of this kinase and enhanced signaling from T cell antigen receptor (table 1).

In red blood cells, where LMPTP is abundant, it has been suggested to dephosphorylate the erythrocyte membrane protein band III [12]. This integral membrane protein is phosphorylated by the Lyn and Syk tyrosine kinases, a modification that leads to reduced binding of several peripheral membrane proteins [13], including the metabolic enzymes phosphofructokinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase and catalase, and elevated glycolytic rates [14]. Dephosphorylation by LMPTP would be expected to have the opposite effect.

In vitro, the two LMPTP isoforms are also active against flavin mononucleotide (FMN), dephosphorylating it to riboflavin [2]. In Chinese hamster ovary cell extracts, LMPTP constitutes most of the intracellular FMN phosphatase activity [15]. LMPTP may be important for respiration and the antioxidant defense of cells by the regulation of the levels of FMN and flavin nucleotide-dependent enzymes, including glutathione reductase. Thus

ACP1 seems to have important functions in both general metabolism and immunological reactions.

We have reported an association of ACP1 with immunological diseases like type 1 diabetes mellitus (T1DM), Crohn's disease (CD) and allergy [16–21] and some data suggest an opposite pattern of association between Th1 and Th2 classes of immunological disease [22] and between sexes [16, 18].

In consideration of the current interest in gender differences in autoimmunity and of the indication of the Task Force on Gender, Multiple Sclerosis and Autoimmunity [23] we reanalyzed the data collected by our group to clarify the role of gender in the association between ACP1 and immunological diseases.

Material and Methods

Samples

The following samples were considered:

Sample 1: Retrospective Survey of a Cohort Identified at Birth (table 2)

We interviewed individuals studied at birth more than 20 years ago. In these newborns, ACP1 phenotype had been determined by starch gel electrophoresis. Out of 400 subjects studied at birth, 48 were still living at the addresses given in the clinical records. We asked these 48 subjects if they had allergic disorders including asthma, rhinitis and atopic eczema/dermatitis syndrome. All subjects with allergic symptoms reported they had been evaluated at least once and had at least one positive prick test. Only some of these subjects agreed to have more than one cutaneous prick test.

Informed consent for participation in the study was obtained for all subjects.

Sample 2: Population-Based Study in Schoolchildren (table 3)

As a part of a project on preventive pediatrics, a random sample of 126 schoolchildren was studied of the white Caucasian population of Viterbo (Latium). The survey was approved by the Sanitary Authority and informed consent was obtained from the parents to include their children in the study. Skin prick test was performed in the absence of any drugs.

Table 2. Interaction between gender and ACP1 concerning their effects on susceptibility to allergy controlled by prick test in a cohort of subjects studied at birth (sample 1)

	Prick test		Proportion of subjects with positive prick test	OR for susceptibility to allergy (females vs. males)
	negative	positive		
<i>Subjects carrying the *A allele</i>				
Females	4	4	50%	2.80
Males	14	5	26%	
<i>Other ACP1 genotypes</i>				
Females	16	0	0%	<0.25
Males	4	1	20%	

Allergic symptoms include asthma, conjunctivitis and rhinitis. Three-way contingency table analysis by a log-linear model: x = allergy; y = gender; z = ACP1 genotype; xyz interaction; p < 0.05. OR = Odds ratio.

Table 4. Interaction between gender and ACP1 concerning their effects on susceptibility to allergic asthma (prick positive) in a case-control study (sample 3)

	Controls	Asthma (prick positive)	OR for susceptibility to allergic asthma (females vs. males)
<i>Subjects carrying the *A allele</i>			
Females	70	27	1.00
Males	96	37	
<i>Other ACP1 genotypes</i>			
Females	94	19	0.47
Males	98	42	

Three-way contingency table analysis by a log-linear model: x = disease; y = gender; z = ACP1 genotype; xyz interaction; p = 0.08. OR = Odds ratio.

Sample 3: Case-Control Study in Asthmatic Children (table 4)

The sample study was composed of 125 children aged from 1 month to 12 years. The criterion for inclusion in the study was a history of two or more episodes of wheezing in the previous 6 months, irrespective of the etiology/pathogenesis of the attack. Functional assays were not performed in all subjects. All children were prick positive.

Informed consent for participation in the study was obtained from the parents.

Table 3. Interaction between gender and ACP1 concerning their effects on prick test positivity in a population-based study (sample 2)

	Prick test		Proportion of subjects with positive prick test	OR for prick test positivity (females vs. males)
	negative	positive		
<i>Subjects carrying the *A allele</i>				
Females	26	10	28%	1.92
Males	25	5	17%	
<i>Other ACP1 genotypes</i>				
Females	25	6	19%	0.34
Males	17	12	41%	

Three-way contingency table analysis by a log-linear model: x = prick test; y = gender; z = ACP1 genotype; xyz interaction; p < 0.05. OR = Odds ratio.

Table 5. Interaction between gender and ACP1 concerning their effects on susceptibility to CD in a case-control study (sample 4)

	Controls	CD	OR for susceptibility to CD (females vs. males)
<i>Subjects carrying the *A allele</i>			
Females	70	7	0.50
Males	96	19	
<i>Other ACP1 genotypes</i>			
Females	94	27	1.56
Males	98	18	

Three-way contingency table analysis by a log-linear model: x = disease; y = gender; z = ACP1 genotype; xyz interaction; p < 0.05. OR = Odds ratio.

Sample 4: Case-Control Study in Adult Subjects with CD (table 5)

We studied 71 patients with CD of the population of Rome admitted consecutively to the Division of Gastroenterology of the S. Camillo Hospital. In all patients, the diagnosis of CD was made correlating clinical symptoms with endoscopic findings, mucosal biopsy studies and radiological evaluation of the small and large intestine. The absence of inflammatory intestinal disease other than CD was based on clinical, serological, microbiological and pathological data. Informed consent for participation in the study was obtained from all patients.

Sample 5: Case-Control Study in Children with T1DM (table 6)

One hundred and eighty-eight consecutive children with T1DM diagnosed at the Department of Pediatrics of the University of Sassari (Sardinia) were studied. The type of diabetes was diagnosed in all subjects according to standard criteria. All the patients came from northern Sardinia; this region is characterized by an ethnically homogeneous population at high risk of T1DM. Informed consent to carry out the present study was obtained either from the patients or from their parents. Mean age at diagnosis was 7.4 years (SE 0.29).

In a previous paper we investigated the effect of the interaction between gender and ACP1 on the age at onset in T1DM [16]. The present paper is focused on the possible effect of the interaction between ACP1 and gender on susceptibility to T1DM. Other previously published studies on these samples are cited by Bottini et al. [18, 20].

Prick Test

Prick tests were performed with a panel of local allergens (produced by Lofarma Allergeni S.p.A., Milan, Italy): *Dermatophagoides pteronyssinus* and *D. farinae*, *Alternaria tenuis*, *Aspergillus fumigatus* (mould), *Poa pratensis* (grass), *Artemisia vulgaris* (composite), *Parietaria officinalis* (Urticaceae), *Olea europaea* (olive), milk albumin, egg white and cat dander.

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. Subjects in whom at least one of the test of allergens gave a significant wheal were considered prick positive.

ACP1 Phenotype and Genotype Determination

In samples 1, 2, 3 and 4 ACP1 phenotype was determined by the classical method of Hopkinson et al. [24] by starch gel electrophoresis on red cell hemolysates. In sample 5, ACP1 genotype was determined by RFLP-polymerase chain reaction (PCR).

ACP1 is a highly polymorphic enzyme controlled by an autosomal locus showing 3 codominant alleles named ACP1*A, *B and *C. The three ACP1 alleles show single base substitutions located at 3 specific sites: ACP1*A and *B alleles differ for 2 base substitutions, a silent C-T transition at codon 41 (exon 3), and an A-G transition at codon 105 (exon 6). The ACP1*C allele differs from the *A and *B alleles by a C-T transition at codon 43 (exon 4). Total genomic DNA was extracted from frozen whole-blood samples collected in Na₂EDTA. All PCRs were set up in 30 µl, 0.2 µM of both primers, 0.1 mM dNTPs, 1.5 mM MgCl₂, 0.5 U of Taq polymerase (AmpliTaQ, Applied Biosystem, Mannheim, Germany), 1× AmpliTaq buffer (PE), and 50 ng of DNA template. The amplification conditions consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, 54°C for 45 s, 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 µM, respectively.

Table 6. Interaction between gender and ACP1 concerning their effects on susceptibility to T1DM in a case-control study (sample 5)

	Controls	T1DM	OR for susceptibility to T1DM (females vs. males)
<i>Subjects carrying the *A allele</i>			
Females	27	25	0.35
Males	18	47	
<i>Other ACP1 genotypes</i>			
Females	33	50	0.96
Males	42	66	

Three-way contingency table analysis by a log-linear model: x = disease; y = gender; z = ACP1 genotype; xyz interaction; p < 0.05.

The C-T transition at codon 43 and the A-G transition at codon 105 generate, respectively, a *CfoI* and a *TaqI* restriction site that, together, were used for PCR-based genotyping [modified from 25]. A 341-bp segment spanning the entire exons 3 and 4 was amplified using primers 263 to 264 as previously described [16]. A 299-bp segment including exon 6 was amplified using primers 267 and 268 [16]. Ten microliters of the 341-bp exon 3 amplicon were fully cleaved by *CfoI* at 37°C for 1 h according to the manufacturer's instructions and then submitted to electrophoresis on 1.8% agarose gels. The digestion created 2 fragments of 255 and 86 bp for the ACP1*A and ACP1*B alleles, while the ACP1*C allele was not cut. Similarly, the 299-bp PCR product was digested by *TaqI* 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 the *C alleles.

In our laboratory classical starch gel phenotype determination and DNA genotype determination gave the same results.

Statistical Methods

Three-way contingency table analysis and combined probability were performed according Sokal and Rohlf [26]. Other statistical tests were carried out according to SPSS programs [27]. The combined probability technique makes it possible to consider a series of separate significance tests on different sets of data to test the same scientific hypothesis. For each test the probability may be low enough to be suggestive but not sufficiently to be statistically significant.

Results

Table 2 shows the relationship between allergy, ACP1 and gender in a cohort of subjects studied at birth. There is a significant interaction among the variables suggesting that the pattern of association between allergy and

ACP1 depends on gender. In subjects carrying the *A allele of ACP1 the proportion of those suffering of allergic manifestations is greater in females than in males, while among subjects carrying other ACP1 types the pattern is reversed and the proportion of allergic subjects is greater in males than in females. Overall a significant increase of allergic manifestations in the presence of *A allele is observed in females only. In females with allergy the proportion of those carrying the *A allele is significantly greater than in females without allergy ($p = 0.006$). This is not observed in males.

Table 3 shows the relationship between prick test positivity, ACP1 and gender in a population-based study. There is a significant interaction among the variables suggesting that the pattern of association between prick test positivity and ACP1 depends on gender. In subjects carrying the *A allele of ACP1, the proportion of those with a positive prick test is greater in females than in males, while among subjects carrying other ACP1 types, the pattern is reversed and the proportion of allergic subjects is greater in males than in females.

Table 4 shows the relationship between asthma, ACP1 and gender in a case-control study. There is a borderline interaction among the variables suggesting that the pattern of association between asthma and ACP1 depends on gender. The impact of gender on atopic dermatitis is different than in asthma. This may explain the relatively low level of significance in this sample as compared to samples 1 and 2 in which only some of the allergic subjects had asthmatic episodes. Odds ratio analysis suggests that while the susceptibility to asthma in subjects carrying the *A allele is similar in males and females, among subjects carrying the other ACP1 types the susceptibility to asthma is greater in males than in females. In females with asthma but not in males the proportion of *A allele carriers is significantly greater than in controls: asthma 58.7% versus controls 42.7% in females ($p = 0.05$) and asthma 46.8% versus controls 49.5% in males ($p =$ not significant). The pattern in subjects positive to one allergen is similar to that observed in those positive to more than one allergen (data not shown).

We also considered a sample of 167 adult women presenting for surgical problems: the proportion of subjects with allergic symptoms with a positive prick test was 36% among carriers of *A allele and 26% among other ACP1 genotypes. Although the difference does not reach the level of statistical significance, this observation is in line with the hypothesis of an increased susceptibility to allergy in women carrying the ACP1*A allele.

Table 7. Relationship between sex ratio (males/females) and ACP1 in classes of immunological diseases

	Carriers of *A allele	Other ACP1 types
Allergy (Th2) samples 1, 2 and 3		
Sex ratio	1.1	2.2
Total, n	88	80
CD (Th1)		
Sex ratio	2.7	0.7
Total, n	26	45
TIDM (Th1)		
Sex ratio	1.9	1.3
Total, n	72	116

Three-way contingency table analysis by a log-linear model: $x = \text{ACP1}$; $y = \text{gender}$; $z = \text{class of disease}$.

Comparison between CD and TIDM: xyz interaction; p value: nonsignificant.

Comparison between Th1 and Th2 classes: xyz interaction; $p < 0.005$.

A cumulative probability analysis of data in tables 2–4 showed a χ^2 of 17.97 with 6 degrees of freedom corresponding to a probability of less than 0.01 for interaction among allergic manifestations, gender and ACP1. This indicates that the relationship between ACP1 and allergy depends on gender: the presence of *A allele seems to make females more susceptible to allergic manifestations than males.

Table 5 shows the relationship between CD, ACP1 and gender in a case-control study. There is a significant interaction among the variables suggesting that the pattern of association between CD and ACP1 depends on gender. Odds ratio analysis suggests that while the susceptibility to CD in subjects carrying the *A allele is greater in males than in females, among subjects carrying other ACP1 types the susceptibility to CD is greater in females than in males. In females with CD the proportion of *A allele carriers is much lower than in controls ($p < 0.025$).

Table 6 shows the relationship between TIDM, ACP1 and gender in a case-control study. There is a significant interaction among the variables suggesting that the pattern of association between TIDM and ACP1 depends on gender. Odds ratio analysis suggests that susceptibility to TIDM in subjects carrying the *A allele is greater in males than in females. In females with TIDM the proportion of those carrying the *A allele is lower than in controls ($p \sim 0.05$).

The pattern of interaction between disease, gender and ACP1 in T1DM is similar to that observed in CD. A cumulative probability analysis of data in tables 5 and 6 showed a χ^2 equal to 11.98 with 4 degrees of freedom corresponding to a probability of less than 0.02 for interaction among this class of immune diseases (Th1), gender and ACP1. This indicates that the relationship between ACP1 and disease depends on gender. The presence of *A allele seems to make females less susceptible to this class of diseases than males.

Table 7 shows the relationship between sex ratio and the presence of ACP1*A allele in different classes of immunological diseases. The pattern of sex ratio is similar in CD and T1DM (Th1 disease) with the sex ratio being higher in carriers of *A allele than in other ACP1 phenotypes. In allergic subjects (Th2 disease) the pattern is opposite to that observed in Th1 diseases and the sex ratio is lower in ACP1 phenotypes carrying the *A allele than in other types.

Discussion

Current interest is focused on gender differences in autoimmunity [23]. Experimental studies indicate that viruses that elicit Th1 or Th2 responses may have a different clinical expression in males and in females [28–30]. In humans different degrees of immune response have been observed between sexes. Clinical disease expression including age at onset, progression and response to treatment may also show gender differences. Sex hormones could have an important role through modulation of immune response. Estrogens seem to enhance immune reactivity at a low level but inhibit it at a higher level [31–34]. Testosterone exerts immunosuppressive effects on autoimmunity [35, 36]. Pituitary hormones may also be important; prolactin and growth hormone may enhance autoimmunity [23].

Genetic factors too have an important role in autoimmunity. It has been suggested that besides major histocompatibility complex (MHC)-linked candidate genes, there are also non-MHC-linked candidate genes involved in autoimmunity [36, 37]. Moreover, there could be unique genes determining susceptibility of a target organ to an autoimmune attack [23]. There is a general consensus on the importance of the elucidation of molecular mechanisms and, in particular, of the signal transduction pathways involved in the gender differences, including age at onset and the course and severity of autoimmune diseases. It has been proposed that sex hormones could affect the

immune system by influencing the signal transduction pathway involved in immune functions [23]. In this context it should be noted that although the hormonal status is age related, the pattern of interaction observed in sample 1 (adults) is similar to that in sample 2 (children).

The present data and the recent observation suggesting an involvement of ACP1 in signaling from the T cell antigen receptor through activation of ZAP-70 [11] suggest that the enzyme affects the immune systems. At present, however, it is not known whether the expression of F and S isoforms of ACP1 in the immune system is influenced by sex hormones.

The fact that the effect of gender on the pattern of association between the class of Th1 diseases and ACP1 is opposite to the pattern observed between allergy (Th2) and ACP1 suggests the existence of differences between sexes concerning the biochemical mechanism leading to Th1 or Th2 differentiation.

In conclusion, low activity ACP1*A allele seems to influence the susceptibility to allergy and autoimmune diseases in the opposite direction depending on gender. At present the mechanism underlying gender differences is unknown. Further investigation in this area could be rewarding.

Acknowledgments

This study was possible thanks to long term friendly cooperation of the following colleagues: Prof. E. Carapella and Prof. R. Rochetti, Policlinico Umberto 1°, Rome; Prof. G. Ronchetti, Ospedale S. Cammillo, Rome; Prof. T. Meloni, Clinica Pediatrica Università di Sassari, Sassari.

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