

# Phosphotyrosine-Protein-Phosphatase and Diabetic Disorders. Further Studies on the Relationship between Low Molecular Weight Acid Phosphatase Genotype and Degree of Glycemic Control

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**ABSTRACT:** We have studied a new sample of 276 NIDDM patients from the population of Penne (Italy). Comparison of the new data with those of 214 diabetic pregnant women from the population of Rome reported in a previous paper has shown that the pattern of association between low molecular weight acid phosphatase genotype and degree of glycemic control is similar in the two classes of diabetic patients.

Among nonobese subjects the proportion of ACP1\*A (the allele showing the lowest enzymatic activity) is lower in diabetic patients with high glycemic levels (mean value greater than 8.9 mmol/l) than in diabetic patients with a low glycemic level (mean value less than 8.9 mmol/l). Among obese subjects no significant association is observed between glycemic levels and ACP1.

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Among nonobese subjects the concentration of f isoform of ACP1 is higher in patients showing a high glycemic level than in patients showing a low glycemic level. No significant difference is observed for s isoform.

**KEYWORDS:** cLMW-PTP, protein tyrosine phosphatase, ACP1, diabetes, glycemic level, diabetes heterogeneity

## INTRODUCTION

Protein tyrosine phosphorylation is implicated in normal and neoplastic cell growth and proliferation and in signal transduction by insulin. The phosphorylation state is balanced by the action of kinases and phosphatases (PTPases) [3,6,8]. Abnormal PTPase regulation has been reported in animals and patients resistant to insulin [1,2,15,16].

Cytosolic low molecular weight (cLMW) acid phosphatase encoded by the highly polymorphic locus ACP1 [5,11,21,22] is a member of the PTPase family and is present in all tissues. All ACP1 genotypes show two main isozymes designated f and s according to their relatively fast or slow anodal electrophoretic mobility and the ratio of their activity is markedly different among genotypes. Significant differences between f and s isoforms have been observed in both enzymatic and molecular properties suggesting that they perform different physiological functions [20].

*In vitro* ACP1 is able to hydrolyse phosphotyrosine containing synthetic peptides of

the human insulin receptor and of Band-3-Protein (B3P) [12,20]. High ACP1 activity may favour high glycemic level through a depression of insulin action. On the other hand since phosphorylation of B3P is associated with increased glycolytic rate through activation of aldolase, phosphofruktokinase and glyceraldehyde-3-phosphate dehydrogenase [13], high ACP1 activity may favour high glycemic level through a decrease of the activity of glycolytic enzymes.

Recent observations in diabetic pregnant women from the population of Rome have shown that women with high glycemic level (greater than 8.9 mmol/l) have a very low proportion of genotypes carrying ACP1\*A, the allele associated with the lowest enzymatic activity. The pattern of association is similar in gestational and pre-existing diabetes (IDDM and NIDDM) [7]. We have now observed a concordant pattern of association between ACP1 and glycemic levels in a sample of NIDDM subjects from another Italian population.

## SUBJECTS AND METHODS

A random sample of 276 NIDDM subjects collected from a population of about 2000 diabetic subjects under care in the Center of Diabetology of a local hospital have been studied in the population of Penne, a small rural town in South-Eastern Italy. The sample includes males and females. The age ranges between 24 and 91 years. Glycemic levels are the values of determinations (in most cases the mean value of two determinations) performed within the trimester preceding the collection of the blood samples. ACP1 typing was performed according to Harris and Hopkinson [9]. Since we have described a positive association of ACP1\*A with severe body mass deviation in obese subjects [18] the analysis has been performed separately considering nonobese or moderately obese subjects (BMI < 30) and clearly obese subjects (BMI ≥ 30). Three way contingency tables were analysed using a log-linear model according to

Table 1  
Proportion of low activity ACP1\*A allele in diabetic subjects in relation to glycemic level and body mass index

glycemic level mmol/L		BMI < 30		BMI ≥ 30	
		≤ 8.9	> 8.9	≤ 8.9	> 8.9
NIDDM subjects (Penne)	proportion of ACP1*A allele	26.4%	15.52%	32.42%	32.26%
	total n° of alleles	250	58	182	62
Diabetic pregnant women (Rome)	proportion of ACP1*A allele	28.40%	14.29%	25.00%	12.50%
	total n° of alleles	324	56	40	8

Three way contingency table analysis by a log linear model

	Subjects with a BMI < 30	Subjects with a BMI ≥ 30
Three way interaction		
Effect of sample on the association between glycemic level and ACP1	N.S.	N.S.
Independence between glycemic level and ACP1	P ≅ 0.01	N.S.
Proportion of variance of glycemic level explained by ACP1 genetic variability in diabetic subjects with BMI < 30		
NIDDM		2.1%
Diabetic women		1.5%

Sokal and Rohlf [19]. In all samples studied ACP1 genotype distribution did not show any significant deviation from Hardy–Weinberg expectation.

## RESULTS

Table 1 shows the data of NIDDM subjects from Penne and of diabetic pregnant women from Rome already reported in a previous paper [7]. Among nonobese subjects the proportion of ACP1\*A allele in diabetic patients with a mean glycemic level greater than 8.9 mmol/l is lower than in patients with a mean glycemic level lower than 8.9 mmol/l. Among obese subjects no significant association is observed between glycemic levels and ACP1. The table also reports a measure of the strength of association in nonobese subjects expressed as proportion of variance of glycemic level explained by ACP1 genetic variability.

Table 2 shows the concentration of f and s ACP1 isoforms according to glycemic level. Concentrations of isoforms were assigned to each ACP1 genotype according to Dissing [4]. Nonobese subjects with severe glucose intolerance show a higher level of f isoform as compared to diabetic subjects with a mean glycemic level less than 8.9 mol/l. No significant difference is observed for s isoform.

Table 2  
f and s ACP1 isoform concentration in diabetic subjects with body mass index < 30

		BMI < 30	
glycemic level mmol/L		≤ 8.9	> 8.9
f isoform concentration	mean	13.30	14.39
	S.E.	0.17	0.34
	t	p = 0.0045	
s isoform concentration	mean	4.88	4.94
	S.E.	0.18	0.40
	t	p = 0.444	

t (one tail probability) refers to comparison of subjects showing a glycemic level ≤ 8.9 mmol/L with those showing glycemic level > 8.9 mmol/L.

No significant effect of sex, age, age at onset and duration of disease on the association between glycemic level and ACP1\*A allele has been observed in our NIDDM sample.

## DISCUSSION

The similarity of the pattern of association between ACP1 and glycemic level in heterogeneous clones of diabetic disorders as gestational diabetes, IDDM and NIDDM, suggests that ACP1 may regulate some mechanism involved in the final steps of the phosphorylation cascade regulating glucose utilisation. Indeed, while an association between ACP1 and glycemic level has been constantly observed, in no sample ACP1 genotype distribution has shown significant differences from that of control population, arguing against the possibility that ACP1 may be “per se” a factor influencing the origin of a diabetic disorder.

Stefani et al. [20] have shown that a synthetic-phosphotyrosine-containing peptide corresponding to the 5-16 sequence of Band-3-Protein is more efficiently hydrolysed by the f component than by the s component of ACP1. Since dephosphorylation of B3P decreases the glycolytic rate [13] the fact that a positive association with glycemic levels has been observed with f isoform only, points to modulation of B3P phosphorylation as a possible mechanism underlying the association between ACP1 and glycemic level.

The specificity of the association with ACP1 isoform and the similarity of the pattern observed in different populations and in different classes of diabetic disorders make it very unlikely that the association may represent a mere chance artefact. Although the possibility that the association may reflect the action of a gene located very near to ACP1 and in linkage disequilibrium with it cannot be excluded at present, the evidence based on the functions of ACP1 and on the different properties of f and s isoforms suggests a direct causal role.

Subjects with NIDDM may show several anomalies such as decrease in insulin receptor number, tyrosine kinase activity, defect in glucose transporter translocation and glycogen-synthesis. About 15% of NIDDM patients have genetic variants of IRS-1 but its functional significance is still unclear. At present, however, none of the primary candidate genes studied has revealed to be a major locus of mutation in NIDDM [10]. Recently a possible pathogenic role of molecules that act as inhibitors of insulin action has been suggested [14]. Genetic variability of PTPases may also have an important role both in susceptibility and in clinical variability of diabetic disorders.

As shown in Table 1 the proportion of variance of glycemic levels explained by ACP1 genetic variability (i.e. the strength of association) is around 1.5% for NIDDM and 2% for diabetic women. Since glycemic control is multifactorial, these figures ought not to be considered meaningless: although ACP1 is not a "major" factor, our data suggest that it may have a respectable position among "background" genes influencing the expression of diabetes. Many PTPases are known at present [6] and it cannot be expected that post-receptorial regulation of insulin action and/or glycolytic rate may depend on ACP1 alone. Although different isoforms and genetic variability probably exist for other PTPases also [6] as far as we know the existence of a genetic polymorphism associated with different concentrations of isoforms and strong differences of total enzymatic activity among genotypes have been demonstrated, up to now, for ACP1 only. This and the basic conservation of enzyme structure during evolution [17] point to an important role of ACP1 in cellular metabolism and call for further studies on its clinical relevance.

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