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Phosphotyrosine protein phosphatases and diabetic pregnancy: an association between low molecular weight acid phosphatase and degree of glycemic control

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Abstract. Low molecular weight acid phosphatase encoded by the highly polymorphic locus ACP1 is a member of the protein-tyrosin phosphatase family (PTPases) which plays an essential role in the control of receptor signalling through phosphotyrosine pathways. Recent experiments have shown that purified rat liver ACP, corresponding to human ACP1, is able to hydrolyze a phosphotyrosine-containing synthetic peptide corresponding to the 1146–1158 sequence of the human insulin receptor, and shows a high affinity for it. This prompted us to analyze the degree of glycemic control in relation to ACP1 genetic variability in a sample of 214 diabetic pregnant women including IDDM, NIDDM and gestational diabetes. The ACP1 genotype was also determined in 482 non-diabetic pregnant women. In diabetic women glycemic levels in the last trimester of pregnancy appear to be significantly associated with the ACP1 genotype, and correlate positively with ACP1 enzymatic activity. The data suggest that quantitative variations of ACP1 may influence the clinical mainifestations of diabetic disorders, and call for further studies on the role of this enzyme in the modulation of insulin-receptor phosphotyrosine pathways.
Key words. Tyrosine phosphatases; ACP1; diabetic pregnancy.

Acid phosphatase controlled by locus 1 on chromosome 2 (ACP1) is a member of a family of low molecular weight acid phosphatases present in human erythrocytes, in rat liver and in other tissues of humans and other animal species. The animal enzymes have sequence similarities with human ACP1[1–8]. Experimental evidence indicates that ACP1 has phosphotyrosine phosphatase (PTPase) activity[1,4,9,10]. Therefore the enzyme may have a role in cellular growth regulation and in modulation of glycolytic rate through the control of receptor activities[1–4,11].

ACP1 activity shows great quantitative differences among genotypes[12,13]. Spencer et al.[12] found the following levels of enzymatic activity, expressed as micromoles of p-nitrophenyl phosphate liberated in 30 minutes per gram of Hb at 37°C: ACP1*A/*A = 122.4; ACP1*A/*B = 153.9; ACP1*B/*B = 188, 3; ACP1*A/*C = 183.6; and ACP1*B/*C = 212.3.

Since the phosphorylation state of critical target proteins, including those of the insulin action pathway, is balanced by the action of kinases and phosphatases[14,15], genetically determined quantitative variations of ACP1 enzymatic activity could influence the clinical pattern of diabetic disorders. In particular, low PTPase activity would contribute to increasing the degree of glycemic control.

Recent experiments on a phosphotyrosine-containing synthetic peptide corresponding to the 1146–1158 sequence of the human insulin receptor has shown that purified isoforms 1 and 2 of rat liver ACP1 are able to hydrolyse this substrate, and show a high affinity for it[16]. In the present note we have investigated a possible relationship between the ACP1 genotype and the degree of glycemic control in diabetic subjects.

Materials and methods

98 pregnant women with insulin-dependent diabetes mellitus (IDDM), 33 with non-insulin-dependent diabetes mellitus (NIDDM), and 83 women with gestational diabetes, living in Rome, were included in the study. Women in whom glucose intolerance developed or was discovered during pregnancy were included in the gestational diabetes group. The sample of IDDM women represents a subset of the patients under control for IDDM in the Diabetology Department of the 1st University of Rome. 482 non-diabetic pregnant women were also studied as controls.

Diabetic pregnant women were taught to check glycemia, glycosuria and ketonuria daily at home by means of a commercial stick test. A full diabetic ap-

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praisal together with an obstetric, clinical, biochemical and biophysical evaluation was performed every 1–2 weeks, according to the time of pregnancy, during brief stays in the hospital. Treatment, adjusted on the basis of the clinical evaluation, was carried out either with diet alone (46% of gestational diabetes) or with diet plus insulin (all preexisting diabetes and 54% of gestational diabetes).

Eight or more blood glucose determinations were carried out during the last trimester of pregnancy. The degree of glycemic control was classified into three categories: (a) mean glycemic level during the last trimester below 6.67 mmol/L; (b) mean glycemic level between 6.67 and 8.90 mmol/L and (c) mean glycemic levels greater than 8.90 mmol/L. Blood glucose was determined by the hexokinase method according to standard procedures.

ACP1 genotype was determined according to Harris and Hopkinson by starch gel electrophoresis[17]. Total enzymatic concentration (μg/ml of packed RBC) was assigned to ACP1 genotypes according to Dissing[13].

The chi squared test of independence, and correlation analyses, were carried out using SPSS programs[18]. A three-way contingency table analysis was performed according to Sokal and Rohlf[19].

Results

Table 1A shows the relation between ACP1 enzymatic activity assigned on the basis of genotype and glycemic level. There is a linear positive correlation between glycemic level and ACP1 activity. The pattern is similar in all types of diabetes. Comparison with non-diabetic pregnant women shows a significantly higher mean ACP1 activity in diabetic women with high and very high glycemic levels.

Table 1B shows the relation between the presence of low activity variants ACP1*A/*A (homozygous for the ACP1*A allele) and ACP1*A/*B (heterozygous genotypes) and glycemic level. In all types of diabetes the proportion of ACP1*A/*A and ACP1*A/*B shows a negative correlation with the glycemic level. Subjects with ACP1*A/*A or *A/*B genotype are less represented in the groups with high and very high glycemic levels as compared to subjects with low glycemic level. Comparison with non-diabetic pregnant women shows a significantly smaller proportion of ACP1*A/*A and *A/*B in diabetic women with high and very high glycemic levels. Three-way contingency table analysis showed that the pattern described is very similar in all types of diabetes.

Table 2 shows the proportion of low activity ACP1 variants in women with gestational diabetes treated with diet alone and with diet plus insulin. Women treated with diet alone show a higher proportion of ACP1*A/*A and ACP1*A/*B compared both to

Table 1. ACP1 activity* and proportion (per cent) of low activity ACP1 variants** in relation to glycemic level*** during the last trimester of pregnancy.

	Diabetic women												Non-diabetic pregnant women
	IDDM			NIDDM			Gestational			All types			
	a	b	c	a	b	c	a	b	c	a	b	c	
A) ACP1 activity	17.9 ± 0.6	18.5 ± 0.6	19.4 ± 0.6	17.7 ± 07	17.9 ± 1.0	20.3 ± 0.0	17.7 ± 0.4	19.3 ± 0.8	19.7 ± 2.3	17.7 ± 0.3	18.6 ± 0.5	19.6 ± 0.5	17.9 ± 0.15
B) Proportion of low activity ACP1 variants	47.5	33.3	29.0	53.6	40.0	0.0	50.1	30.1	33.3	50.0	33.9	25.0	46.7
Number of subjects	40	33	25	19	10	4	67	13	3	126	56	32	482

*Values are means $\pm$ SEM; **ACP1 *A/*A and ACP1 *A/*B genotypes; ***a = mean glycemic level < 6.67 mmol/L; b = mean glycemic level $\geqslant 6.67$ and $\leqslant 8.90$ mmol/L; c = mean glycemic level > 8.90 mmol/L.

A) *ACP1 activity:* correlation between ACP1 activity and degree of glycemic control (all types of diabetes), $p < 0.005$; comparison between the whole group of diabetic women and non-diabetic pregnant women; glycemic level < 6.67 mmol/L, N.S.; glycemic level $\geqslant 6.67$, $p < 0.01$.

B) *Proportion of low activity ACP1 variants:* effect of type of diabetes on the association between glycemic control and ACP1 (three way contingency table analysis by log linear model), N.S.; association between glycemic control and ACP1 (Chi square), all types of diabetes, $p < 0.015$; correlation between degree of glycemic control and proportion of *A/*A and *A/*B (Mantel–Haenszel test), all types of diabetes, $p < 0.002$; comparison with non-diabetic pregnant women (Chi square), all types of diabetes: glycemic level < 6.67, N.S.; glycemic level $\geqslant 6.67$, $p < 0.01$.

Table 2. Proportion of low activity ACP1 variants (*A/*A and *A/*B) in women with gestational diabetes treated with diet alone and with diet plus insulin.

	Women with gestational diabetes		Non-diabetic pregnant women
	treated with diet alone	treated with diet plus insulin	
Proportion of *A/*A and *A/*B subjects	55.3%	40.0%	46.7%
Total n	38	45	482

women treated with diet plus insulin and non-diabetic pregnant women; the difference, however, does not attain the level of statistical significance. Since women with gestational diabetes treated with diet alone generally have a less severe glucose intolerance, this observation also points to a negative correlation between the proportion of ACP1*A/*A and ACP1*A/*B and glycemic level.

Discussion

Low activity variants of ACP1 seem to represent a protective factor against severe hypoglycemia during pregnancy. The association between the degree of glycemic control and ACP1 has been observed in all classes of diabetic disorders. The same pattern of association has also been observed, considering the indication to treat with insulin as being an index of severity in gestational diabetes. It therefore appears unlikely that the association may represent merely a chance sampling artefact.

The glycemic level is the end result of endogenous and exogeneous factors, depending on both the basic severity of the disease and on the therapeutical efforts. ACP1 genotype is a characteristic determined at the time of zygote formation, and as such cannot depend either on the severity of disease or on the therapy provided. On the other hand, ACP1 might influence the severity of disease, intensity of therapy required, or both. The present data indicate that at least one of these mechanisms is operating. The relationship between ACP1 and type of treatment in gestational diabetes supports the hypothesis of a direct effect of ACP1 on the severity of disease but does not exclude other effects. A prospective study might contribute to clarifying the problem.

Diabetes might influence ACP1 phenotypic activity as well; this seems worth investigating. We would stress, however, that such possibility has no bearing on the interpretation of data in table 1A. Since ACP1 activity was assigned on the basis of genotype, the association represents a statistical relation of glycemic level with ACP1 genotype and can be interpreted in the direction 'ACP1' genotype → glycemic level' and only in this direction.

An association does not represent the demonstration of a casual relationship. At present, the effect of other genes near ACP1 and in linkage disequilibrium with it cannot be excluded. However, the described association cannot be considered 'random'. In fact, we searched for it on the basis of a priori knowledge of ACP1 functions which suggested relevance to diabetic disorders, and we found in our data a pattern of relationships consistent with the properties of the enzyme. Therefore, we are inclined to consider ACP1 as causal in the association.

Protein-tyrosine phosphorylation is implicated in normal and neoplastic cell growth and proliferation, and in signal transduction by insulin. In most cell types insulin initiates its metabolism-promoting effects by activating the intramolecular autophosphorylation of specific tyrosine residues of a subunit of the receptor, thereby enhancing the tyrosine kinase activity of the receptor itself towards other protein substrates[20]. The phosphorylation state is balanced by the action of kinases and phosphatases. Insulin also increases protein-tyrosine phosphatase (PTPase) expression, which suggests a feed-back regulation of signalling through the insulin action pathway[11, 14, 15, 21, 22]. The PTPase family includes a large number of enzymes, suggesting an important role in the control of cell growth and signal transduction. Such diversity is probably required for an accurate regulation of the complex and highly integrated metabolic pathways of eucaryotic cells[22].

Genetically determined low-activity variants of PTPases may enhance insulin effects on metabolism, favoring glycemic control. The negative association between ACP1 activity and the degree of glycemic control in diabetic pregnancies supports this hypothesis and is in line with the data showing a high affinity of ACP1 isoforms for a synthetic sequence of the human insulin receptor[16]. Our observations should encourage further studies in this direction. ACP1 is a polymorphic PTPase showing genetically determined quantitative variations. This may represent an advantage in the analysis of the relation between PTPase activity and biological effects in experimental systems.

Since pregnancy induces quantitative alterations of PTPases similar to those elicited by diabetes[23], it is not possible to exclude that the association described is specific of/or enhanced by diabetic pregnancies. A very preliminary analysis of a new sample of 274 NIDDM subjects that we are studying in the population of Penne seems to confirm a relation between degree of glycemic control and ACP1 genotype: the proportion of ACP1*A/*A and *A/*/B genotypes is 48.2% in subjects with a good glycemic control and 40% in those with unsatisfactory control. HbAlc level shows a similar relation with ACP1 genotype.

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