Type 2 Diabetes and the Genetics of Signal Transduction: A Study of Interaction Between Adenosine Deaminase and Acid Phosphatase Locus 1 Polymorphisms

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Acid phosphatase locus 1 (ACP1) is a highly polymorphic enzyme that has an important role in flavoenzyme activity and in the control of insulin receptor activity and band 3 protein phosphorylation status. Adenosine deaminase (ADA) is a polymorphic enzyme that catalyses the irreversible deamination of adenosine to inosine and has an important role in regulating adenosine concentration. Based on the hypothesis that ACP1 counteracts insulin signaling by dephosphorylating the insulin receptor and that adenosine has an anti-insulin action, we reasoned that low ACP1 activity (low dephosphorylating action on insulin receptor) when associated with high ADA activity (low adenosine concentration) would result in a cumulative effect towards an increased glucose tolerance. On the contrary, high ACP1 activity when associated with low ADA activity would result in a cumulative effect towards a decreased glucose tolerance. A total of 280 adult subjects with type 2 diabetes from the population of Penne (Italy) were studied. There was a nonsignificant trend toward an increase in the proportion of subjects with the complex type with high ACP1 activity and low ADA activity (ie, *B/*B; *A/*C; *B/*C; *C/*C//ADA*1/*2 and *2/*2) in type 2 diabetes relative to that observed in newborn infants from the same population. High ACP1 activity/low ADA activity joint genotype was positively associated with high glycemic levels and with high body mass index (BMI) values. Low ACP1 activity/high ADA activity joint genotype was also positively associated with dyslipidemia. These findings suggest that both ACP1 and ADA contribute to the clinical manifestations of type 2 diabetes and probably also have a marginal influence on susceptibility to the disease. Both additive and epistatic interactions between the 2 systems seem to be operative.

WE HAVE RECENTLY reported that subjects with type 2 diabetes having an adenosine deaminase (ADA) genotype with low enzymatic activity show a strong tendency to high body mass index (BMI).[1] Subjects with low activity ACP1 (acid phosphatase locus 1 also named cytosolic low–molecular-weight protein-tyrosine phosphatase [cLMWPTP]) genotypes show a tendency toward low glycemic levels[2,3] and to high BMI values.[4]

We hypothesized that ACP1 activity counteracts insulin signalling by dephosphorylating the insulin receptor and that adenosine has an anti-insulin action.[5,6] Additionally, previous studies have shown that ADA and ACP1 interact at a biochemical level.[7] On the basis of this evidence, we have now re-evaluated our earlier data, suspecting that possible interactions between the 2 systems may influence their separate effects on the clinical manifestations of type 2 diabetes.

ACP1 is a polymorphic enzyme controlled by a locus on chromosome 2 showing 3 common codominant alleles: ACP1*A, ACP1*B, and ACP1*C. These 3 alleles are associated with different enzymatic activity.[8] Two functions have been suggested for ACP1: flavin-mononucleotide-phosphatase and tyrosine phosphatase activity.[9-11] Catalysing the conversion of flavin-mononucleotide (FMN) to riboflavin, ACP1 may have a role in regulating the cellular concentration of flavin-adenine-dinucleotide (FAD), flavo-enzyme activity and energy metabolism. As phosphotyrosine phosphatase, the enzyme may have an important role in modulation of glycolytic rate through the control of insulin receptor activities and of band 3 protein phosphorylation status.[2-4,7,8,10,12]

ADA is a polymorphic enzyme present in all mammalian tissues.[13] It is controlled by a locus with 2 codominant alleles ADA*1 and ADA*2 located on the long arm of chromosome 20,[14] that are associated with different enzymatic activity. ADA catalyses the irreversible deamination of adenosine to inosine. Red blood cells (RBC) are in equilibrium with freely diffusing adenosine[15] pointing to an important role for this enzyme in the regulation of adenosine concentration.

Adenosine, a purine nucleoside present in plasma and other extracellular fluids, is an important local hormone (like prostaglandins) regulating blood flow, neurotransmission, physiology of smooth muscle, and platelet aggregation. Current interest is focused on a wide variety of effects produced by adenosine via activation of cell surface adenosine receptors.[5-18] Recent studies have shown that adenosine counteracts insulin action in the liver by activating A2B receptors.[6] On the adipocyte, adenosine seems to facilitate insulin action. The adenosine deaminase complex protein[19] (ADPC) is identical to CD26, a T–cell-activating antigen and with a glycoprotein present in epithelial cells of various tissues. Recent data suggest that ADA and CD26 are colocalized on the T-cell surface, but not inside cells.

Cell expressing ADA and CD26 on the surface are much more resistant to the inhibitory effects of adenosine. These data suggest that ADA on the cell surface is involved in an important immunoregulatory mechanism by which released ADA

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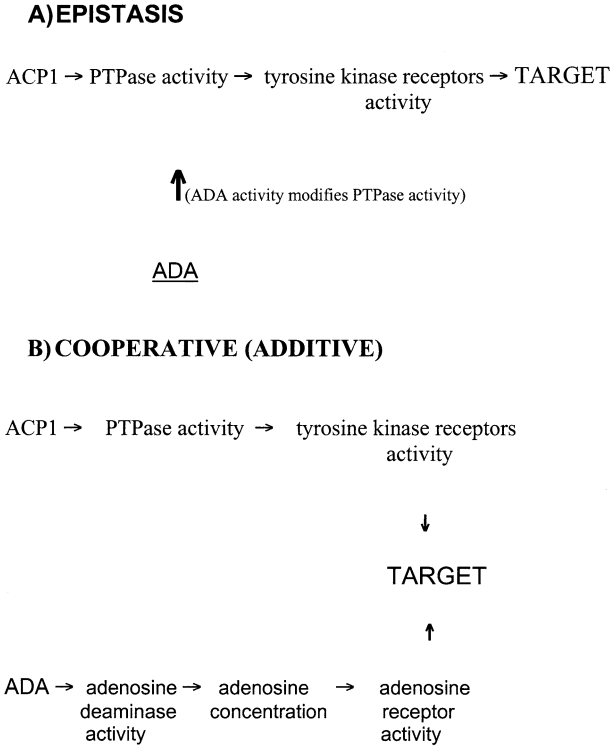

Fig 1. Possible modes of interaction between ADA and ACP1.

binds to cell surface CD26, and this complex is capable of reducing the local concentration of adenosine.[20]

ACP1 is modulated by various substances including purines. Lucarini et al[7] have shown that ACP1 activity is influenced by adenosine and ADA genotype. ACP1 activity in carriers of ADA*2 allele is generally lower than in homozygotes for ADA*1 allele. There is also an interaction between ADA and ACP1 genotypes concerning their effects on ACP1 activity. ACP*A/*A carrying ADA*2 allele shows a 37% lower activity than ACP1*A/*A with ADA*1/*1 genotype; ACP1*A/*B carrying the ADA*2 allele shows a 13% lower activity than ACP1*A/*B with ADA*1/*1 type, while for other ACP1 types, no significant differences have been observed between carriers of ADA*2 and subjects with ADA*1/*1 genotype. As a result, a very low ACP1 activity is observed in individuals having ACP1*A/*A genotype and carrying ADA*2 allele. Because ACP1 may have a significant role in modulating glycolytic rate and energy metabolism, its genetic variability could influence physiologic and pathologic processes. Effects related to low ACP1 activity (ie, *A/*A and *A/*B genotypes) could be modified in individuals carrying the ADA*2 allele. Effects of adenosine receptor activation have been reported both on protein kinases[21,22] and on tyrosine phosphatases.[23,24] Collectively, these data suggest interactions between ADA and ACP1 both at physiological and biochemical levels.

Table 2. Cooperative Effects of ACP1 and ADA Genotypes

Symbol	Joint Genotype		Effects on Glucose Tolerance
L/H	Low ACP1 activity	→	Increased glucose tolerance
	High ADA activity	→	Increased glucose tolerance
L/L	Low ACP1 activity	→	Increased glucose tolerance
	Low ADA activity	→	Decreased glucose tolerance
H/H	High ACP1 activity	→	Decreased glucose tolerance
	High ADA activity	→	Increased glucose tolerance
H/L	High ACP1 activity	→	Decreased glucose tolerance
	Low ADA activity	→	Decreased glucose tolerance

NOTE. ACP1 genotypes are divided into 2 classes: low activity (*A/*A and *A/*B) and high activity (*B/*B, *A/*C, *B/*C, and *C/*C). ADA genotypes are divided into 2 classes: high activity (ADA *1/*1) and low activity (ADA1*/*2 and ADA *2/*2).

The rationale for the analysis presented in this report is depicted in Fig 1 and Tables 1 and 2. High ADA activity (ADA *1/*1 genotype), which is associated with a low concentration of adenosine and low activity of the adenosine receptor should result in increased glucose tolerance.[5] On the other hand, low ADA activity (ADA *1/*2 and *2/*2 genotypes) should result in decreased glucose tolerance. Low PTPase activity (ACP1*A/*A and *A/*B genotypes) is associated with low dephosphorylating activity at the insulin receptor and with increased glucose tolerance.[2,3] Similarly, ACP1 genotypes showing medium-high activity (*B/*B,*A/*C,*B/*C, and *C/*C) have a high dephosphorylating activity at the insulin receptor and decreased glucose tolerance.

The possible modes of ADA-ACP1 interaction are shown in

Table 1. Mechanisms by Which ADA and ACP1 May Act at Receptorial Level

ACP1 *A/*A and *A/*B	→	Low PTPase activity	→	Low dephosphorylating activity on tyrosine kinase receptors	→	High activity of tyrosine kinase receptors	→	Increased glucose tolerance
Other ACP1 genotypes	→	High PTPase activity	→	High dephosphorylating activity on tyrosine kinase receptors	→	Low activity of tyrosine kinase receptors	→	Decreased glucose tolerance
ADA *1	→	High adenosine deaminase activity	→	Low concentration of adenosine	→	Low activity of adenosine receptors	→	Increased glucose tolerance
ADA *2	→	Low adenosine deaminase activity	→	High concentration of adenosine	→	High activity of adenosine receptors	→	Decreased glucose tolerance

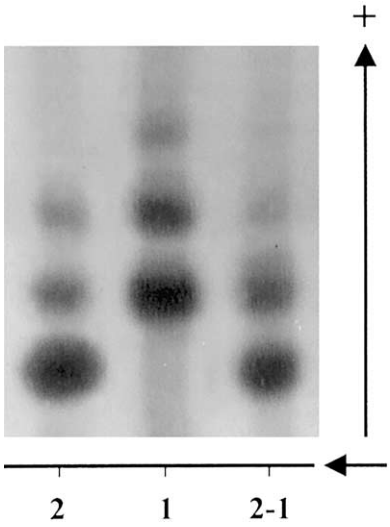

Fig 2. The electrophoretic patterns of ADA phenotypes.

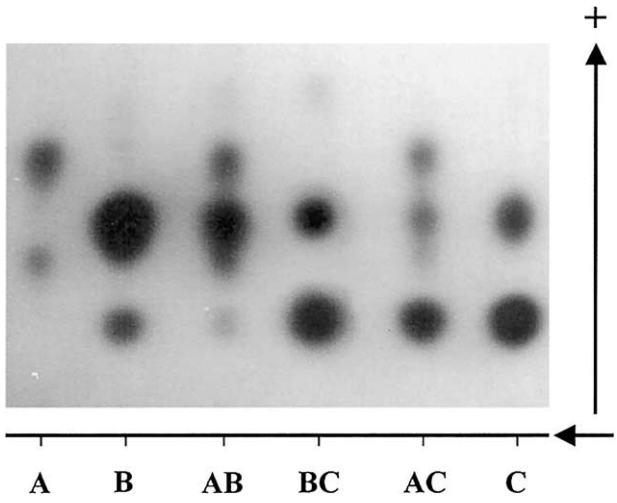

Fig 3. The electophoretic pattern of ACP1 phenotypes.

Fig 1. Assuming an additive model (cooperative effect of ACP1 and ADA genotypes), our analysis is directed to test the hypotheses depicted in Table 2. Low ACP1 activity when associated with high ADA activity would result in a cumulative effect of increasing glucose tolerance. On the other hand, high ACP1 activity when associated with low ADA activity would result in a cumulative effect towards a decreased glucose tolerance. In the other situations, there would be a balance between opposite effects of ACP1 and ADA resulting in an intermediate level of glucose tolerance. It is very likely that epistatic effects are also operating that produce deviations from the results predicted by the purely additive model in Table 2.

SUBJECTS AND METHODS

A total of 280 subjects with type 2 diabetes from the population of Penne, Italy were studied. Subjects were a random sample from a population of about 2,000 subjects under care at the Center of Diabetology of the local Hospital. The sample includes 128 males and 147 females, aged 24 to 91 years. Further details are reported in a previous report.[1]

The ADA genotype was determined by starch gel electrophoresis on RBC hemolysates according to Spencer et al.[13] Inosine produced at the sites of ADA activity is converted in hypoxanthine in the presence of nucleoside phosphorylase and phosphate. The hypoxanthine is then oxidized by the action of xanthine oxidase and during this reaction the tetrazolium salt MTT is reduced in the presence of phenazine methosulphate, to a blue insoluble formazan. In ADA*1/*1 type, there are 3 regularly spaced components which exhibit decreasing staining intensity in order of their anodal electrophoretic mobilities. In ADA*2/*2 type, there are also 3 isozymes and their relative intensities and relative electrophoretic mobilities are very similar to those of the ADA1 pattern. The difference between ADA*1/*1 and ADA*2/*2 is that the ADA*2/*2 pattern is appreciably slower than the ADA*1/*1 pattern. The pattern exhibiting 4 isozymes, designated ADA*2/*1 has the appearance of a mixture of ADA*1/*1 and ADA*2/*2 pattern (see Fig 2).

The ACP1 phenotype was determined by starch gel electrophoresis on RBC hemolysates according to Harris and Hopkinson.[25] The acid phosphatase pattern is revealed by a solution of phenolphtalein diphosphate: the addendum of ammonium solution reveals the area where phenolphtalein has been liberated in the areas of gel where ACP1 activity is present. In European populations, the presence of 3 common alleles *A,*B, and *C determines the occurrence of 6 phenotypes: A, AB, B, AC, BC, and C. Each of the homozygous A, B, and C phenotypes are composed of 2 fractions, f and s, corresponding to a fast and slow component of electrophoretic pattern. Heterozygous phenotypes have a pattern corresponding to a mixture of homozygous types (see Fig 3). In the last 2 years in our laboratory, determination of ADA and ACP1 genotypes has been performed routinely on DNA. Comparison of classical with DNA methods has not shown differences in genotypic classification.

Glycemic and glycosylated hemoglobin (HbA_{1c}) levels are the fasting values of determinations (in most cases the mean value of 2 determinations) performed within the trimester preceding the collection of blood samples. Blood lipid levels were considered abnormal if triglyceridemia was above the range of 130 to 200 mg/100 mL and/or lipoproteinemia was above the range of 40 to 165 mg/mL. Chi square, logistic regression, and variance analysis have been performed using SPSS statistical package (SPSS, Chicago, IL).[26] Three-way contingency tables have been analyzed by a log-linear model according to Sokal and Rohlf.[27] Because of random missing data, total numbers of subjects in the various tables are slightly different among variables considered.

Table 3. Sample Distribution of Some Relevant Variables

Continous variables	Means	SE
Blood glucose (mg/dL)	135.4	2.3
HBA_{1c} (% of total Hb)	7.6	0.1
Age at sampling (yr)	66.3	0.6
Age at onset (yr)	54.4	0.6
Duration of disease (yr)	11.9	0.5
Body mass index	29.5	0.3
Categorical variables	**% Proportions**	**SE**
Males	47%	3.0%
Presence of dyslipidemia	27%	2.8%
Treatment with insulin	16%	2.0%

NOTE. Total number of patients, 280. HbA_{1c} was determined in 271 subjects.

Table 4. Percent Distribution of Joint ACP1/ADA Genotype in Type 2 Diabetes Subjects and in Newborn Infants From the Same Population

	Joint ACP1/ADA Genotype				
	L/H	L/L and H/H	H/L	Total no.	Comparison H/L *v* Others
Newborns	37.7%	55.1%	7.2%	374	
Type 2 diabetes	35.3%	53.5%	11.3%	275	$P < .10$

NOTE. Genotypes have been grouped into 3 classes: L/H (low ACP1 activity/high ADA activity), L/L plus H/H (low ACP1 activity/low ADA activity and high ACP1 activity/high ADA activity), and H/L (high ACP1 activity/low ADA activity). See also scheme in Table 2.

RESULTS

Table 3 shows the sample distribution of some relevant clinical variables. Table 4 compares the distribution of joint ACP1-ADA genotype subdivided into 3 classes according to the scheme in Table 2. The proportion of subjects with the joint type associated with medium-high ACP1 activity and low ADA activity (ie, *B/*B; *A/*C; *B/*C; *C/*C//ADA*1/*2 and *2/*2) is slightly higher in type 2 diabetes than in newborn infants from the same population, although this difference was nonsignificant ($P < .1$). Table 5 analyzes the effect of ACP1 and ADA on glycemic level, HbA_{1c}, BMI, and dyslipidemia. Three-way contingency table analyses were performed by a log linear model on categorical variables. Significant 3-way interactions were observed for glycemia and BMI, suggesting the presence of an epistatic interaction between ACP1 and ADA influencing these variables. No significant interaction was observed for HbA_{1c} and dyslipidemia. The relationship of glycemic and HbA_{1c} levels with the joint ADA ACP1 type was analyzed with parametric statistics in Table 6. In carriers of ADA*2 allele, there is a significant positive correlation of both variables with ACP1 activity.

Table 7 shows the distribution of joint ACP1-ADA genotypes (subdivided into 3 classes according to the scheme of Table 2) in relation to glycemic level, BMI, and dyslipidemia. The H/L genotype (high ACP1 activity/low ADA activity) is positively associated with high glycemic levels and with high BMI values. L/H genotype (low ACP1 activity/high ADA activity) is positively associated with dyslipidemia.

Table 8 shows 2 logistic regression analyses in which the joint ACP1-ADA type, classified according to the scheme in Table 2, is considered the dependent variable. Assuming as dependent variable the dichotomy (H/L) versus (other classes), glycemia and BMI, but not dyslipidemia, produce a significant contribution to separation into the 2 classes. Assuming as a dependent variable, the dichotomy (L/H) versus other classes),

Table 5. The Effect of ACP1 and ADA on Glycemic Level, HbA_{1c} BMI and Dyslipidemia

		ADA		
	ACP1	ADA 1/1	Carriers of ADA^2 allele	Statistical Analysis*
Glycemia				
≤120 (A)	*A/*A and *A/*B	40	10	X = ADA, Y = ACP1, Z = Glycemic level
	Other genotypes	53	7	x y z interaction
>120 and ≤160 (B)	*A/*A and *A/*B	37	9	A *v* B *v* C: $P < .02$
	Other genotypes	48	12	(A + B) *v* C: $P = .001$
>160 (C)	*A/*A and *A/*B	20	1	
	Other genotypes	26	12	
HbA_{1c}				
1st quartile (A)	*A/*A and *A/*B	25	6	X = ADA, Y = ACP1, Z = Glycemic level
	Other genotypes	31	4	x y z interaction
2nd and 3rd quartile (B)	*A/*A and *A/*B	49	10	A *v* B *v* C: NS
	Other genotypes	61	14	(A + B) *v* C: NS
4th quartile (C)	*A/*A and *A/*B	23	3	
	Other genotypes	30	11	
BMI				X = ADA, Y = ACP1, Z = BMI
BMI ≤ 30	*A/*A and *A/*B	42	12	x y z interaction: $P < .005$
	Other genotypes	74	9	
BMI > 30	*A/*A and *A/*B	54	8	
	Other genotypes	52	22	
Dyslipidemia				X = ADA, Y = ACP1, Z = Dyslipidemia
Absent	*A/*A and *A/*B	61	14	x y z interaction: NS
	Other genotypes	101	22	
Present	*A/*A and *A/*B	34	6	
	Other genotypes	29	9	

Abbreviation: NS, not significant.

*Three-way contingency table analysis by a log-lin model.

Table 6. Glycemic and HbA_{1c} Levels in Relation to ADA and ACP Genotypes in NIDDM Subjects

	ACP1 Genotype										
	*A/*A and *A/*B			*B/*B			*A/*C, *B/*C, *C/*C			Statistical Variance Analysis *P*	Analysis of Linearity *P*
	Mean	SE	no.	Mean	SE	no.	Mean	SE	no.		
Glycemic level											
ADA *1*1	135.9	4.1	93	135.1	4.3	83	134.1	5.9	33	NS	NS
Carriers of ADA *2	116.5	7.0	18	147.7	8.2	21	166.9	19.8	8	.006	.02
HbA_{1c} level											
ADA *1*1	7.6	0.2	92	7.4	0.2	78	7.7	0.3	33	NS	NS
Carriers of ADA *2	7.1	0.3	17	7.7	0.3	19	8.6	0.7	8	.102	.035

Abbreviation: NS, not significant.

dyslipidemia, but neither glycemia nor BMI, produce a significant contribution to the separation into the 2 classes.

A 1-way analysis of variance (independent joint ACP1-ADA type subdivided into 3 classes) has been performed on glycemia, BMI, age at onset, duration of disease, age, and HbA_{1c}. Glycemia ($P = .01$ for H/L *v* other) and BMI ($P = .012$) showed significant differences among the joint ACP1-ADA types. The results for HbA_{1c} were similar to those for blood glucose, but did not reach the level of statistical significance ($P = .12$ for H/L *v* others) (data not shown). Chi square tests of independence between ACP1-ADA joint type (into 3 classes) and sex, dyslipidemia and treatment with insulin were also performed. Dyslipidemia showed a statistically significant association with ACP1-ADA joint genotype ($P = .016$ for LH *v* others) (data not shown).

A variance analysis was performed, treating as dependent glycemia and as independent dyslipidemia, joint ACP1-ADA type, sex, age at onset and BMI. Joint ACP1/ADA genotype and age at onset have shown a strong effect on glycemic level ($P < .005$). Sex and interaction between sex and ACP1/ADA type also show a significant effect ($P < .025$), while an interaction between ACP1/ADA joint type and BMI shows a borderline significant effect ($P = .065$) (data not shown).

A variance analysis considering BMI as dependent and dyslipidemia, ACP/ADA joint genotype, sex, age at onset of disease, and glycemia as independent has shown that ACP1/ADA joint genotype has a significant effect on BMI ($P < .05$). An interaction between ACP1/ADA genotype and sex has shown a borderline significant effect on BMI ($P = .057$) (data not shown).

We performed a logistic regression considering dyslipidemia as dependent and glycemia, BMI, joint ACP1/ADA type, age at onset of disease, duration of disease, sex, and HbA_{1c} as independent. ACP1/ADA joint type produced a significant effect ($P < .01$) and duration of disease a moderate effect ($P < .05$) on dyslipidemia (data not shown).

DISCUSSION

The present analysis suggests a cooperative interaction between ADA and ACP1 systems that influences the clinical manifestations and to a lesser extent the susceptibility/resistance to type 2 diabetes. However, our analyses also indicate that epistatic interactions are also operative. Further studies comparing diabetics with controls of the same age and sex are

Table 7. Percent Distribution of Joint ACP1/ADA Genotype in Type 2 Diabetes in Relation to Glycemic Level, BMI, and Dyslipidemia

	Joint ACP1/ADA Genotype				Statistical Analysis (chi-square test of independence)
	L/H (A)	L/L and H/H (B)	H/L (C)	Total No.	
Glycemia					
≤160	36.0%	56.1%	7.9%	214	A *v* B *v* C: $P < .02$
>160	33.9%	45.8%	20.3%	59	(A + B) *v* C: $P < .01$
BMI					
≤30	30.7%	62.8%	6.6%	137	A *v* B *v* C: $P < .00$
>30	39.7%	44.1%	16.2%	136	(A + B) *v* C: $P = .01$
Dyslipidemia					
Absent	30.1%	58.1%	11.1%	198	A *v* B *v* C: $P = .16$
Present	43.6%	44.9%	11.5%	78	A *v* (B + C); $P < .05$

NOTE. Genotypes have been grouped in 3 classes according to the scheme in Table 1.

Table 8. Logistic Regression Analysis Considering as Dependent the Joint ACP1-ADA Genotype and as Independent Glycemia, BMI, and Dyslipidemia

Dependent	(H/L) *v* (other classes)
Independent	Glycemia, BMI, dyslipidemia
Initial chi-square	10.637; 3 *df*; $P = .0139$
Final chi-square	10.578; 2 *df*; $P = .005$
	Variables in the equation
	Glycemia $P = .0209$
	BMI $P = .0227$
Residual chi-square	0.059; 1 *df*; $P = .8079$
Dependent	(L/H) *v* (other classes)
Independent	Glycemia, BMI, dyslipidemia
Initial chi-square	6.541; 3 *df*; $P = .0881$
Final chi-square	5.826; 1 *df*; $P = .0158$
	Variables in the equation
	Dyslipidemia $P = .0154$
Residual chi-square	0.712; 2 *df*; $P = .7005$

NOTE. Backward stepwise procedure.

necessary to clarify the possible effect of ADA-ACP1 joint genotype in susceptibility/resistance to type2 diabetes.

Concerning the effect of ACP1 on glycemia, the observations are in line with the results forecast on the basis of our observations of the association with ACP1 genotype[2,3] and of experimental data on the action of ACP1 on the insulin receptor.[28] On the basis of the effects of activation of adenosine receptors on glucose tolerance, the effects of ADA on glycemia are also in line with the expected results.[5]

Concerning the effects of ADA on BMI, the observations reported here are consistent with the results that were predicted on the basis of the effects of adenosine receptors activity on adiposity in diabetic disorders.[5] Regarding the ACP1 findings, previous observations suggest that low activity genotypes (*A/*A and *A/*B) favor obesity in type 2 diabetes; however these observations have also shown an epistatic interaction with dyslipidemia. Thus the discrepancy between expected and observed association between ACP1 and BMI might be due to these noncooperative interactions.

The physiological basis of epistatic interactions between ADA and ACP1 and of their effects on the clinical manifestations of diabetes could be complex. Some observations however may give hints concerning the underlying mechanisms. ACP1 activity is dependent on ADA activity (see Introduction). Activation of adenosine receptors of adipocytes shows an effect on glucose uptake opposite to that seen in the muscle.[5] Adipocyte acid phosphatase is 99% identical to ACP1 and dephosphorylates the adipocyte lipid binding protein,[29] pointing to a possible direct involvement of this PTPase in lipid disposal.

A major limitation of case-control studies in multifactorial inheritance is represented by spurious associations generally due to confounding ethnicity. The people of Penne are the descendents of an ancient italic population, and in the last 20 centuries, no invasion or significant immigration from other areas has been recorded. Thus the population can be considered ethnically homogeneous.

Another drawback of case-control studies is represented by the high false positive rate in most cases resulting from the low prior probability for a causal relationship between the genetic polymorphism and the disease investigated. In the present study, we have examined 2 systems for which biochemical functions,[1,8] experimental data,[5,28] and previous observations in humans[2,3] point to a high prior probability of involvement in diabetic disorders.[30]

As pointed out by Sing et al,[31] important steps in the analysis of genetic architecture of multifactorial variables like common diseases are the product of the joint distribution of genetic and environmental causal agents and the ascertaiment of combinations of genes and environmental causes responsible for determining variation of risk of disease Epistatic genetic interactions may also play an important role: because of such interactions, it is possible that the effect of a single gene might be missed because we are detecting only marginal effects.[31] Our study suggests that both ACP1 and ADA contribute to clinical manifestations of type 2 diabetes and probably also to its susceptibility, and that there are both cooperative and epistatic interactions between the 2 systems.

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