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The effect of genetic variability on the correlation between blood glucose and glycated hemoglobin levels

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Abstract

The disturbing results of recent clinical trials aimed to control cardiovascular risk of diabetes by aggressive control of blood glucose prompted us to analyze the effect of genetic variability of 2 polymorphic enzymes abundant in red blood cells on the correlation between blood glucose and glycated hemoglobin (Hb). Two hundred eighty subjects with type 2 diabetes mellitus were studied. Adenylate kinase locus 1 (AK_1) and acid phosphatase locus 1 were determined. Correlation between blood glucose and glycated Hb was determined for phenotypes of the 2 systems. The correlation between blood glucose and glycated Hb is higher in carriers of AK_1^*2 allele than in subjects with $AK_1 1$ phenotype. The highest coefficient is observed in acid phosphatase locus 1 phenotypes with the highest enzymatic activity; and the lowest, in phenotypes with the lowest activity. Effects of sex, blood glucose level, age, age at onset, and duration of disease have been also considered. Our data are in agreement with recent observation in healthy subjects suggesting a role of genetic factors on glycated Hb level. If glycation of structural and functional protein is dependent not only on blood glucose level but also on genetic factors, these factors could have an important role in the susceptibility and clinical course of diabetes.

1. Introduction

The disturbing results of recent clinical trials aimed to control cardiovascular risk of diabetes by aggressive control of blood glucose [1] prompted us to examine the correlation between blood glucose and glycated hemoglobin (Hb) in a sample of subjects with type 2 diabetes mellitus studied about 15 years ago [2].

Currently, the best measure of blood glucose level in clinical practice is glycated Hb that measures the percentage of Hb molecules that have undergone glycation.

In the present note, we have examined the correlation between blood glucose and glycated Hb in relation to 2 polymorphic enzymes that are abundant in red blood cells: adenylate kinase locus 1 (AK_1) and acid phosphatase locus 1 (ACP_1).

1.1. Adenylate kinase locus 1

Adenylate kinase locus 1 catalyzes the reaction adenosine triphosphate (ATP) + adenosine monophosphate (AMP) ↔ 2 adenosine diphosphate (ADP) and belongs to the family of nucleotide monophosphate kinases. Growing evidence indicates that AK-phosphotransfer dynamics through AMP signaling regulates many intracellular and extracellular signaling processes including hormonal secretion, nuclear transport, energetic of cell cycle, DNA synthesis, and the developmental programming [3].

Besides AK_1, 4 other AKs have been identified within the nucleotide monophosphate kinase family that show sequence similarities to AK_1. The gene controlling AK_1 is located on chromosome 9q32. AK_1 is located in the cytosol and is polymorphic in humans showing 2 common alleles: AK_1^*1 and AK_1^*2. $AK_1 1$ phenotype is more active than $AK_1 2$-1 and $AK_1 2$ [4].

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1.2. Acid phosphatase locus 1

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 white populations, there are 6 genotypes attributed to the presence of 3 codominant alleles, ACP_1^*A, ACP_1^*B, and ACP_1^*C, at an autosomal locus [5]. The enzyme is composed of 2 isoforms, F and S, that have different molecular and catalytic properties [6,7].

Two separate functions have been attributed to ACP_1, that is, phosphoprotein tyrosine phosphatase and flavin mononucleotide phosphatase [7]. Acid phosphatase locus 1 dephosphorylates a negative phosphorylation site of the ZAP-70 tyrosine kinase in T cells [8]. This event leads to increased activation of this kinase and enhanced signaling from T cell antigen receptor.

Acid phosphatase locus 1 is involved in the negative modulation of insulin signal transduction [9] and is able to in vitro dephosphorylate the adipocyte lipid binding protein (ALBP) [10]. The ALBP belongs to a family of binding lipid proteins present in various isoforms in many human tissues. In adipose tissue, ALBP is phosphorylated on Tyr19 after insulin stimulation; and this phenomenon seems to impair its fatty acid binding ability [11]. In this tissue, the double activity of ACP_1 (on insulin receptor transduction and ALBP phosphorylation) could partly compensate each other.

Insulin signal transduction is modulated by other cytosolic tyrosine phosphatases that act with higher affinity than ACP_1 [12]. Two transmembrane phosphatases in adipocytes that are responsible for the dephosphorylation of ALBP with high affinity have been isolated [13]. The effect of ACP_1 may become evident in the regulation of metabolic signaling only in pathologic situations when other control systems usually acting with higher affinity are failing.

Stefani et al [14] have studied the dephosphorylation of tyrosine phosphorylated synthetic peptides by rat liver phosphotyrosine protein phosphatase isoenzymes A and B that correspond to the human ACP_1 F and S isoforms and have found differences in kinetic parameters of A and B isoenzymes for insulin receptor and B3P phosphorylated peptides.

2. Material and methods

2.1. Subjects

We have re-examined the data on 280 subjects with type 2 diabetes mellitus from the population of Penne (Italy). These patients have been considered in a previous study [2] and were a random sample of a population of about 2000 subjects under care at the Centre of Diabetology of the local hospital. Penne is a rural town located in the eastern side of Central Italy. This homogenous population is the descendant of an old Italic population called *Vestini*. The total number of subjects shown in the tables is not always the same because of some random missing value for the variables considered.

The patients are controlled in the Centre of Diabetology according to a regular schedule. In occasion of the control, blood glucose and glycated Hb levels are measured (more than 9 hours since the last meal). Blood sample for determination of genetic markers are also obtained.

2.2. Laboratory analysis

Serum glucose concentration was measured by the automated Roche/Hitachi Cobas C501 (Basel, Switzerland) system based on enzymatic reaction with exochinase. Glycated Hb was determined using the Menarini Diagnostics (Florence, Italy) HA-8160 automated equipment based on inverse exchange cationic chromatography.

The ACP_1 phenotype has been determined by starch gel electrophoresis on red blood cell hemolysates according to Spencer et al [5]. The acid phosphatase pattern is revealed by a solution of phenolphthalein diphosphate: the addendum of ammonium solution reveals the area where phenophtalein has been liberated in the areas of gel where ACP_1 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 is 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.

The AK_1 phenotype was determined by starch gel electrophoresis of hemolysate [4]. Samples were examined at pH 7. The insert was made from Whatman (London, England) no. 3 filter paper. After electrophoresis, the gels were sliced and then covered with a 0.75% agar solution at 45°C made in 0.1 mol/L Tris buffer pH 8 and containing glucose 10 mmol/L, magnesium chloride 20 mmol/L, ADP 1 mmol/L, nicotinamide adenine dinucleotide phosphate (NADP) 0.4 mmol/L, phenazine methosulfate 0.012%, tetrazolium salt 0.012%, glucose-6-phosphate dehydrogenase 0.04 U/mL and hexokinase 0.08 U/mL. The agar was allowed to set, and then the gel was incubated at 37°C for 2 hours.

At the sites of AK activity, ADP is converted into AMP and ATP. The ATP reacts with glucose in the presence of hexokinase to produce ADP and glucose-6-phosphate. This is oxidized to 6-phosphogluconate by glucose-6-phosphate dehydrogenase with concomitant reduction of NADP. The reduced NADP in the presence of phenazine methosulfate causes the reduction of tetrazolium salt to give a blue-colored insoluble formazan, which is thus deposited at the sites of AK activity. In white populations, 3 distinct types of electrophoretic pattern are recognized, referred to as $AK_1$1, $AK_1$2-1, and $AK_1$2, corresponding to the

presence of 2 codominant alleles: AK_1^*1 and AK_1^*2 at an autosomal locus.

2.3. Statistical analysis

Correlation analyses were carried out by SPSS (Chicago, IL) program [15]. The square of r (coefficient of correlation) measures the strength of correlation, that is, the proportion of glycated Hb variance explained by blood glucose variance. Significance of difference between correlation coefficient has been calculated according to Snedecor and Cochran [16].

3. Results

Table 1 shows demographic and clinical parameters of the sample study. Mean blood glucose and mean glycated Hb are higher in women than in men.

Table 2 shows the distribution of AK_1 and ACP_1 phenotypes in type 2 diabetes mellitus patients and in controls. The ACP_1 phenotype distribution in women shows a significant difference compared with healthy controls because of a decrease of A and an increase of BA and CA phenotypes. Phenotype distribution in men is in agreement with Hardy-Weinberg expectation ($P = .602$). In women, the distribution differs from Hardy-Weinberg expectation ($P = .008$) mainly because of a defect of A and an excess of CA phenotypes. The distribution of AK_1 phenotypes does not show appreciable differences between sexes and between diabetic subjects and controls.

Table 1
Demographic and clinical data on the sample study.

	Mean	Frequency	SE	Significance of difference (t test)
Blood glucose (mg/dL)				
Men	128.7		2.76	.009
Women	140.7		3.55	
Glycated Hb (% of total Hb)				
Men	7.28		0.15	.025
Women	7.76		0.15	
Age at sampling (y)				
Men	65.6		0.92	.313
Women	66.8		0.75	
Duration of disease (y)				
Men	11.07		0.68	.141
Women	12.50		0.68	
Body mass index				
Men	29.1		0.35	.163
Women	29.9		0.45	
Age at onset of diabetes				
Men	54.6		0.95	.847
Women	54.3		0.91	
				χ^2 test
Sex				
Men		47.0%		
Women		53.0%		
Presence of dyslipidemia				
Men		26.2%		.448
Women		27.6%		
Treatment with insulin				
Men		12.2%		.065
Women		19.6%		

Table 2
Distribution of ACP_1 and AK_1 phenotypes in type 2 diabetes mellitus

	Men (a)	Women (b)	Controls (blood donors)
ACP_1			
A	8.4%	3.4%	8.6%
B	42.0%	40.5%	43.9%
C	0.0%	0.0%	0.2%
BA	32.8%	40.5%	31.6%
CA	6.1%	8.1%	3.4%
CB	10.7%	7.4%	12.2%
n	131	148	417
Comparisons	χ^2	*df*	*P*
a vs b	5.417	4	.247
a vs c	1.828	4	.767
b vs c	13.921	4	.007
AK_1			
1	93.1%	94.6%	94.-3%
2-1	6.9%	5.4%	5.7%
2	0.0%	0.0%	0.0%
n	131	148	389
Comparisons	χ^2	*df*	*P*
a vs b	0.261	1	.610
a vs c	0.086	1	.768
b vs c	0.009	1	.920

Table 3 shows blood glucose and glycated Hb levels in AK_1 and ACP_1 phenotypes. No significant difference is observed among ACP_1 phenotypes either for blood glucose or for glycated Hb. Blood glucose is significantly higher in $AK_1$2-1 than in $AK_1$1 subjects. The $AK_1$2-1 subjects show a higher level of glycated Hb as compared with the $AK_1$1 subjects, but this difference is not statistically significant.

Table 4 shows the correlation between blood glucose and glycated Hb.

In the whole sample, the correlation is highly significant; however r^2 value is 0.333, indicating that only 33% of

Table 3
Blood glucose and glycated Hb levels in ACP_1 and AK_1 phenotypes

	Blood glucose		Glycated Hb	
	Mean	SE	Mean	SE
ACP_1 genotypes				
A + BA	132.1	3.5	7.5	0.2
B + CA	138.0	3.4	7.5	0.2
CB	133.4	7.3	7.8	0.3
Variance analysis	*P* = .469		*P* = .763	
AK_1 phenotypes				
$AK_1$1	133.9	2.3	7.5	0.1
$AK_1$2-1	156.9	11.8	8.2	0.4
t test	*P* = .015		*P* = .121	

Table 4
The correlation between blood glucose and glycated Hb

	Correlation coefficient (r)	P	r^2
Whole sample	0.577	<.001	0.333
Men	0.521	<.001	0.271
Women	0.587	<.001	0.344
Blood glucose ≤ median	0.083	.335	0.007
Blood glucose > median	0.518	<.001	0.268
Controlling for age, age at onset, duration of disease	0.547	<.001	0.299

glycated Hb variance is explained by blood glucose variance. Sex does not influence significantly the correlation. No correlation is practically present at low blood glucose level. Age, age at onset, and duration of disease do not influence appreciably the correlation coefficient.

Table 5 and Fig. 1 show the effect of AK_1 phenotype on the correlation between blood glucose and glycated Hb levels. A very strong correlation is observed in subjects carrying the AK_1^*2 allele (P = .003 for difference between $AK_1$1 [r = 0.546] and $AK_1$2-1 [r = 0.906]). The difference between $AK_1$1 and $AK_1$2-1 is present in men and women. Age, age at onset, and duration of disease do not influence the difference between AK_1 phenotypes. The analysis that was carried out by Spearman ρ nonparametric test also has given similar results: r_s = 0.605 for $AK_1$1 (P = .000) and r_s = 0.907 (P = .000) for $AK_1$2-1. We have also performed a correlation analysis between blood glucose and glycated Hb in subjects with glucose level greater than 130 m g/dL (median value). This has given the following results: r = 0.499 (P = .000) for $AK_1$1 and r = 0.854 for $AK_1$2-1 (P = .001). This indicates that the difference in correlation between AK11 and $AK_1$2-1 is not due to the higher mean glucose level observed in $AK_1$2-1 as compared to $AK_1$1 subjects.

Table 6 and Fig. 2 show the effect of ACP_1 phenotype on the correlation between blood glucose and glycated Hb. The value of correlation increases with ACP_1 activity. In low-activity phenotypes, 24.7% of variance of glycated Hb is explained by blood glucose variance, whereas in high-activity phenotypes, 54.5% of glycated Hb variance is explained by blood glucose variance. The effect of ACP_1 on the correlation between the 2 parameters is not influenced by age, age at onset, and duration of disease (data not shown).

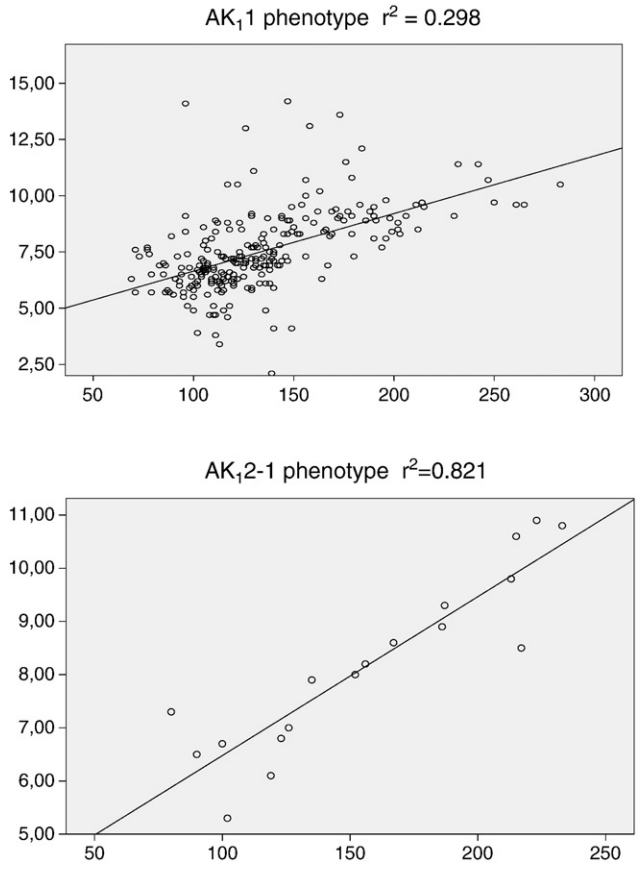

Fig. 1. The correlation between blood glucose (x-axis) and glycated Hb (y-axis) levels according to AK_1 polymorphism.

4. Discussion

The value of correlation coefficient between blood glucose and glycated Hb found in our cases is similar to that found in type 2 diabetes mellitus by Monami et al [17] and indicates that only about one third of glycated Hb

Table 5
The effect of AK_1 phenotype on the correlation between blood glucose and glycated Hb

	Correlation coefficient (r)	P	r^2	n
$AK_1$1 phenotype	0.546	<.001	0.298	253
$AK_1$2-1 phenotype	0.906	<.001	0.821	18

The table shows that the correlation coefficient between blood glucose and glycated Hb is higher in $AK_1$2-1 than in $AK_1$1 phenotype. Significance of difference between correlation coefficients : $AK_1$1 vs $AK_1$2-1 phenotypes, P = .003.

Table 6
The effect of ACP_1 phenotype on the correlation between blood glucose and glycated Hb

	Correlation coefficient (r)	P	r^2	n
A and B/A (low activity)	0.497	<.001	0.247	117
B and C/A (medium activity)	0.619	<.001	0.383	128
C/B and C (high activity)	0.738	<.001	0.545	25

The ACP_1 phenotypes have been grouped according to enzymatic activity. The table shows that the correlation coefficient between blood glucose and glycated Hb increases with ACP_1 enzymatic activity. Significance of difference between correlation coefficients: low vs high ACP_1 activity, P = .07.

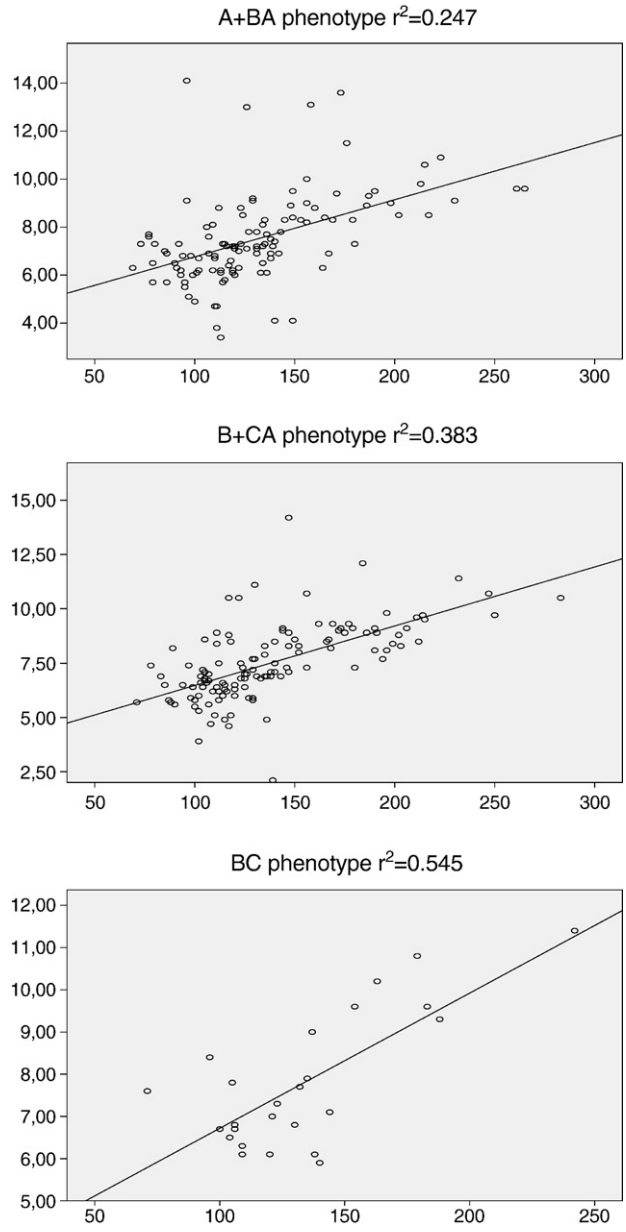

Fig. 2. The correlation between blood glucose (x-axis) and glycated Hb (y-axis) levels according to ACP_1 polymorphism.

variance is explained by blood glucose variance, suggesting that there are other factors that influence the level of glycated Hb and probably of other proteins, too. The recent study of Paré et al [18] showing that genetic factors influence glycated Hb level in nondiabetic individuals is in favor of this hypothesis. A recent study has shown a higher value of correlation between mean glucose and mean glycated Hb levels when measured for a very long period [19]. Other recent studies, however, have shown an effect of race and of viral infection on the correlation between serum glucose and glycated Hb [20,21].

The lack of Hardy-Weinberg equilibrium in the distribution of ACP_1 phenotypes in women with type 2 diabetes mellitus and the difference observed with respect to controls might be due to the influence of ACP_1 activity on insulin action and in turn to susceptibility to diabetes. Indeed, low ACP_1 activity found in A phenotype may favor insulin action, whereas high activity or CA phenotype may have an opposite effect favoring insulin resistance.

The effect of genetic variability of the enzymes investigated is present at high blood glucose concentration only: this suggests that the difference in activity between phenotypes becomes evident only when the metabolic system controlling Hb glycation is near the saturation of its capacity.

It should be stressed that, in the present article, we have observed differences among phenotypes concerning the strength of correlation between blood glucose and glycated Hb. A correlation exists within all phenotypic classes, but it is more or less strong depending on the phenotypes.

Epidemiologic, biochemical and experimental studies point to an important role of ACP_1 in glycide metabolism [2,7,14,22]. Although not statistically significant, the results of the present study are in agreement with all previous data suggesting an unfavorable effect of high ACP_1 activity on glucose tolerance.

As far as we know, studies on the possible relationship between AK_1 polymorphism and glucose metabolism have not been carried out. The difference in glucose level between $AK_1$1 and $AK_1$2/1 phenotypes observed in our cases should encourage further investigation on the role of this enzyme on susceptibility and clinical course of type 2 diabetes mellitus.

Presently, the best measure of blood glucose control is considered glycated Hb because the direct determination of blood glucose provides an indication of current blood glucose level, whereas glycated Hb reflects the level over a course of months. Glycated Hb level, however, depends only in part on blood glucose: other unknown factors have a relevant role.

Hyperglycemia can alter vascular structures and functions in several ways. Glucose permanently bound to structural and functional molecules can modify the elasticity of arteries; it may also influence negatively oxygen delivery and nitric oxide in the cell, contributing to cardiovascular complication of diabetes.

It is likely that most diabetic complications are related to glycation of structural and enzymatic proteins. Therefore, if glycation is dependent not only on glucose level but also on genetic factors, at comparable levels of blood glucose, in the presence of these factors, higher glycation level of proteins could be attained leading to clinical disease and more severe complications.

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