Vol.3, No.2, 77-81 (2011)
doi:10.4236/health.2011.32014

Adenylate kinase locus 1 genetic polymorphism and type 2 diabetes

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Received 9 August 2010; revised 14 February 2011; accepted 18 August 2011

ABSTRACT

AK_1 catalyzes the reversible reaction ATP+AMP ⇔ 2ADP thus contributing to the regulation of relative concentration of these important nucleotides. Intracellular ATP is a storage of energy for cellular processes, moreover extracellular ATP together with ADP, AMP and adenosine are critical signalling molecule for sending messages to nearby cells acting on P1 and P2 receptors. AK_1 shows a genetic polymorphism and recently our group has shown that the correlation between blood glucose and glycated haemoglobin in T2D is dependent on AK_1 phenotype. In the present paper we have carried further studies on the relationship between AK_1 phenotypes and T2D. Possible interactions with ABO blood groups and ACP1 polymorphism have also been investigated. We have re-examined the data on 280 subjects with type 2 diabetes from the White population of Penne (Central Italy). 384 consecutive healthy newborns from the same population have been also studied. A three way contingency table analysis was carried out according to Sokal and Rohlf and other statistical analyses by SPSS programs. T2D patients with $AK_1$2-1 phenotype have higher values of blood glucose level and glycated haemoglobin and an increased tendency to dyslipidemia and retinopathy. In addition there is an interaction of AK_1 with ABO blood groups and with ACP_1 polymorphism. The different activity between AK_1 phenotypes could influence the relative concentration of ATP, ADP, AMP and adenosine with important effects on metabolic activity thus explaining the association of AK_1 with clinical manifestation of T2D.

Keywords: AK; T2D; Glycemia; ATP; Glycated Haemoglobin

1. INTRODUCTION

Adenylate kinase (AK) is an ubiquitous enzyme that catalyzes the nucleotide phosphoryl interconversion ATP + AMP ⇔ 2ADP. The products of this reaction are involved in the regulation of many cellular function and relationship. Intracellular ATP is a storage of energy for cellular processes. The energy from extracellular ATP is used for sending messages to nearby cells.

At first, extracellular ATP was seen simply as a neurotransmitter, subsequently was studied the mechanism of interactions between extracellular ATP signalling with other signalling systems outside the cells named ectoATPases. This large family of AK enzyme removes from ATP, one by one, its phosphates producing adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine that have different effect on several cells by binding themselfes to P2 family (for ADP, AMP) and to P1 (for adenosine) receptors [1].

The family of AK enzymes includes seven genes, AK_1-AK_7, with each other different functions,molecular weight and kinetic property. The network of these enzymes are distributed throughout intracellular compartments, interstitial space and body fluids to regulate energetic and metabolic signaling circuits and to fasten an efficient economy of cell energy, signal communication and stress response.Mutations in AK_1, AK_2 or AK_7 genes have been found associated with hemolytic anemia, reticular dysgenesis and ciliary dyskinesia [2].

Adenylate kinase locus 1 (AK_1) belongs to AK family and plays an important role in the synthesis of nucleotides requested for many metabolic functions. It is present in the cytosol of skeletal muscle, brain, and erythrocyte. The enzyme is polymorphic and shows three phenotypes with different activity, in the order $AK_1$1 > $AK_1$2-1 > $AK_1$2 corresponding to the presence of two codominant alleles, AK_1*1 and AK_1*2 at an autosomal

locus on chromosome 9. Rare alleles AK_1*3, AK_1*4 and AK_1*5 have been also referred.

AK_1 was identified because of its association with a rare genetic disorder causing nonspherocytic hemolytic anemia where a mutation in the AK_1 gene was found to reduce the catalytic activity of the enzyme and the replacement of Arg-128 with Trp [3].

Our group has recently shown that the correlation between blood glucose and glycated haemoglobin in T2D is dependent on AK_1 phenotype. In addition, blood glucose is significantly higher in $AK_1$2-1 than in $AK_1$1 subjects [4]. This prompted us to study in more details the relationship between AK_1 and T2D. We have also considered possible interactions of AK_1 with ABO blood groups that is linked to AK_1 [5] and with ACP_1 that is associated with clinical manifestation of T2D [6].

2. MATERIAL AND METHODS

We have re-examined the data on 278 subjects with type 2 diabetes from the population of Penne (Italy) and have studied 384 consecutive healthy newborns from the same population. T2D patients have been considered in a previous study [6] and were a random sample of a population of about 2000 subjects under care at Centre of Diabetology of the local Hospital. Penne is a rural town located in Eastern side of Central Italy. This homogenous population are the descendant of an old Italic population called "Vestini". The total number of subjects shown in the tables are not always the same due to some random missing value for the variables considered.

The patients have been controlled in the Centre of Diabetology according to a regular schedule. In occasion of the control blood glucose and glycated haemoglobin levels have been measured (more than 9 hours since the last meal). Blood sample for determination of genetic markers were also obtained. Written informed consent was obtained from patients and from mothers of newborns to participate to this study that was approved by the Institutional Review Board.

2.1. Laboratory Analysis

Serum glucose concentration was measured by the automated Roche/Hitachi cobas C501 system based on enzymatic reaction with exochinase. Glycated haemoglobin was determined using the Menarini Diagnostics HA-8160 automated equipment based on inverse exchange cationic chromatography.

AK_1 phenotype was determined by starch gel electrophoresis of haemolysate [7]. Samples were examined at pH7. The insert were made from Whatman, n˚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 M tris buffer pH 8 and containing glucose 10 mM, magnesium chloride 20 mM, adenosine diphosphate (ADP) 1 mM, nicotinammide adenine dinucleotide phosphate (NADP) 0.4 mM, phenazine methosulphate (PMS) 0.012%, tetrazolium salt (MTT) 0.012%, glucose-6-phosphate dehydrogenase (G6PD) 0.04 units/ml and hexokinase 0.08 units/ml. The agar was allowed to set and then the gel incubated at 37˚C for two 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 (G6P), this is oxidized to 6-phosphogluconate by G6PD with concomitant reduction of NADP. The reduced NADP in the presence of PMS causes the reduction of MTT to give a blue-coloured insoluble formazan, which is thus deposited at the sites of AK activity. In Caucasian populations three distinct types of electrophoretic pattern are recognized referred as $AK_1$1, $AK_1$2-1 and $AK_1$2 corresponding to the presence of two codominant alleles: AK_1*1 and AK_1*2 at an autosomal locus.

ACP_1 phenotype has been determined by starch gel electrophoresis on red blood cell haemolysates according to Spencer *et al.* [8]. 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 three common alleles *A, *B and *C determines the occurrence of six phenotypes with enzymatic activity increasing in the order: A < AB < B< AC < BC < C. Each of the homozygous A, B, and C phenotypes are composed of two 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.

2.2. Statistical Analysis

Statistical analyses have been carried out by SPSS program [9]. Three way contingency table analysis has been performed according to Sokal and Rohlf [10].

3. RESULTS

Clinical and demographic data in subjects with T2D and in healthy newborns are shown in **Table 1**.

In T2D the frequency of $AK_1$2-1 is slightly greater in comparison to healthy newborns but the difference is not statistically significant (see **Table 2**). The data suggest that Ak_1 may not be an important factor primarily involved in the susceptibility to type 2 diabetes.

The distributions of blood glucose and glycated Hb are shown in **Table 3**. Both parameters show a greater value in $AK_1$2-1 than in $AK_1$1 but only for blood glucose

Table 1. Clinical and demographic data of the samples studied.

	Mean	S.E.	%Proportion	Total n°
T2D				
Age (yrs)	66	0.59		280
Age at onset of disease (yrs)	54	0.65		275
Duration of disease (yrs)	11.9	0.48		269
BMI	29.5	0.29		277
Male proportion			47%	279
Dyslipidemia			26.8%	276
Healthy Newborns				
Male proportion			47.6%	424
Birthweight (g)	3348.3	22.0		417
Gestational age (wks)	39.7	0.06		411

Table 2. Distribution of AK_1 phenotypes in T2D and in healthy newborns.

	$AK_1$1	$AK_1$2-1	Total n°
T2D subjects			
	93.5%	6.5%	278
Healthy newborns			
	94.3%	5.7%	384
Chi square test of independence			
	χ^2	df	p
	0.054	1	0.816

Table 3. Blood glucose and glycated haemoglobin levels according to AK_1 phenotype in T2D.

	Blood glucose		Glycated Hb	
	Mean	S.E.	Mean	S.E.
$AK_1$1	133.9	2.3	7.51	0.11
$AK_1$2-1	156.9	11.8	8.18	0.39
T-test for differences between means				
	p = 0.015		p = 0.121	

the difference is statistically significant.

The proportion of subjects with dyslipidemia and retinopathy is greater in $AK_1$2-1 than in $AK_1$1 but the difference does not reach the level of statistical significance (see **Table 4**).

In T2D the proportion of AK2-1 phenotype is very high in B blood group and very low in A group. The difference between A and B blood groups is highly significant (O.R. = 10.173 C.I. 2.151-39.163). No significant association has been observed between ABO and AK_1 phenotypes in healthy newborns (see **Table 5**).

Table 6 shows in T2D subjects the distribution of blood glucose and glycated Hb in relation to the joint ABO-AK_1 phenotypes. Both parameters in A and O subjects show higher values in those carrying the $AK_1$2-1 phenotype than in those carrying the $AK_1$1 phenotype while in B subjects there is a slight tendency to a reduction of both parameters in $AK_1$2-1phenotype as compared to Ak1 phenotype.

Figure 1 displays the relationship between serum glucose level and ACP1 enzymatic activity in $AK_1$1 and $AK_1$2-1 subjects with T2D. In $AK_1$1 phenotype the concentration of serum glucose is positively correlated with ACP1 activity while in $AK_1$2-1 phenotype the association follows an opposite pattern.

4. DISCUSSION

The present data show that T2D patients with $AK_1$2-1 phenotype have higher values of blood glucose level and glycated haemoglobin and have an increased tendency to dyslipidemia and retinopathy. In addition there is an interaction of AK_1 with ABO blood groups and ACP_1 polymorphism.

AK_1 belongs to AK family and, at the sites of AK_1 activity, ADP is converted into AMP and ATP. The reaction is reversible thus contributing to the regulation of relative concentration of these important nucleotides [1]. Extracellular ATP plays a physiological role in the maintenance of glucose homeostasis by regulating insulin secretion [11]. ATP is able to stimulate the release of pancreatic insulin modulating glucose transport via GLUT1 acting on P2 purinergic ionotropic (P2X) and P2 metabotropic (P2Y) receptors through a mechanism that involves beta-cell metabolism and a rise of intracellular calcium. P2Y receptors are deficient in fibroblasts from T2D patients [12].

Table 4. Prevalence of dyslipidemia and retinopathy in T2D subjects according to AK_1 phenotype.

	Dyslipidemia			Retinopathy		
	% of subjects with dyslipidemia	Total n°		% of subjects with retinopathy	Total n°	
$AK_1$1	26.0%	258		15.5%	258	
$AK_1$2-1	38.9%	18		27.8%	18	
Chi square test of independence						
	χ^2	df	p	χ^2	df	p
	1.431	1	0.232	1.938	1	0.152

Table 5. Distribution of the joint ABO-AK_1 phenotypes in T2D and in healthy newborns.

	ABO phenotypes			
T2D subjects				
	A	B	AB	O
Proportion of $AK_1$2-1 phenotype	2.7%	21.9%	0.0%	6.2%
Total n°	112	32	4	130
Chi square test of independence				
	χ^2	df	p	
A vs B vs AB vs O	15.497	3	0.001	
A vs B vs O	15.012	2	0.0005	
A vs B	11.378	3	0.0007	
O.R. = 10.173(B vs A/$AK_1$2-1 vs $AK_1$1)				
95% C.I.			2.151-39.163	
Healthy newborns				
Proportion of $AK_1$2-1 phenotype	6.8%	9.7%	0.0%	4.5%
Total n°	162	31	12	178
Chi square test of independence				
	χ^2	df	p	
A vs B vs AB vs O	2.458	3	0.483	
A vs B vs O	1.653	2	0.437	
A vs B	0.036	1	0.849	
O.R.=1.471(B vs A/$AK_1$2-1 vs AK1)				
	95% C.I.		0.027-6.230	
Three way contingency table analysis by a log linear model				
x = ABO (A vs B);				
y = AK_1 ($AK_1$1 vs $AK_1$2-1);				
z = sample (newborns vs T2D)				
	G	df	p	
xyz interaction	4.177	1	0.042	
ODDS ratio analysis (B vs A)/(T2D vs newborns)				
$AK_1$1 phenotype	O.R. = 1.236	95%C.I. 0.656-2.330		
$AK_1$2-1 phenotype	O.R. 8.55	95% C.I. 0.99-92.86		

An increase in the AMP/ATP ratio activates adenine monophosphate activated protein kinase (AMPK) that regulates glycolysis, glucose uptake, lipid oxidation, fatty acid synthesis, cholesterol synthesis and gluconeogenesis. [13,14]. The AMPK channelopathies are present in hyperinsulinemia, neonatal diabetes mellitus and are a risk factor for the aetiology of T2D [15,16].

Table 6. Blood glucose and glycated Hb in T2D according to the joint ABO-AK_1 phenotype.

		ABO phenotype		
Blood glucose				
		A	B	O
$AK_1$1				
	Mean	129.5	141.4	135.3
	S.E.	3.6	8.2	3.3
AK2-1				
	Mean	164.7	139.1	169.5
	S.E.	34.3	15.2	20.0
Significance of difference between means (t-Student)(p)		N.S.	N.S.	N.S.
Glycated Hb				
$AK_1$1				
	Mean	7.31	8.15	7.55
	S.E.	0.19	0.36	0.15
$AK_1$2-1				
	Mean	8.57	7.35	8.75
	S.E.	1.15	0.59	0.54
Significance of difference between means (t-Student) (p)		N.S.	N.S.	0.042

The differences in enzymatic activity between AK_1 phenotypes could influence the relative concentration of ATP, ADP, AMP and adenosine influencing metabolic parameters and contributing to explain the association with clinical manifestation in T2D. Further investigation in this area could be rewarding. Our research is in line with the recent interest on genetic variants that through the regulation of insulin secretion by β-cells could be involved in the pathogenesis and clinical manifestations of type 2 diabetes. [17].

Genetic variability of ABO blood groups substances that are important components of cell membrane structure may influence AK_1 ecto-enzyme activity thus explaining the associations reported in **Tables 5** and **6**.

In general high activity of ACP_1 is associated with high blood glucose level. Low AK_1 activity modifying the ratio among ATP, ADP and AMP may influence the effect of ACP_1 activity on blood glucose resulting in lower glucose level in carriers of high ACP_1 activity genotypes.

5. CONCLUSION

Low adenylate kinase activity associated to $AK_1$2-1 phenotype influencing the relative concentration of ATP,

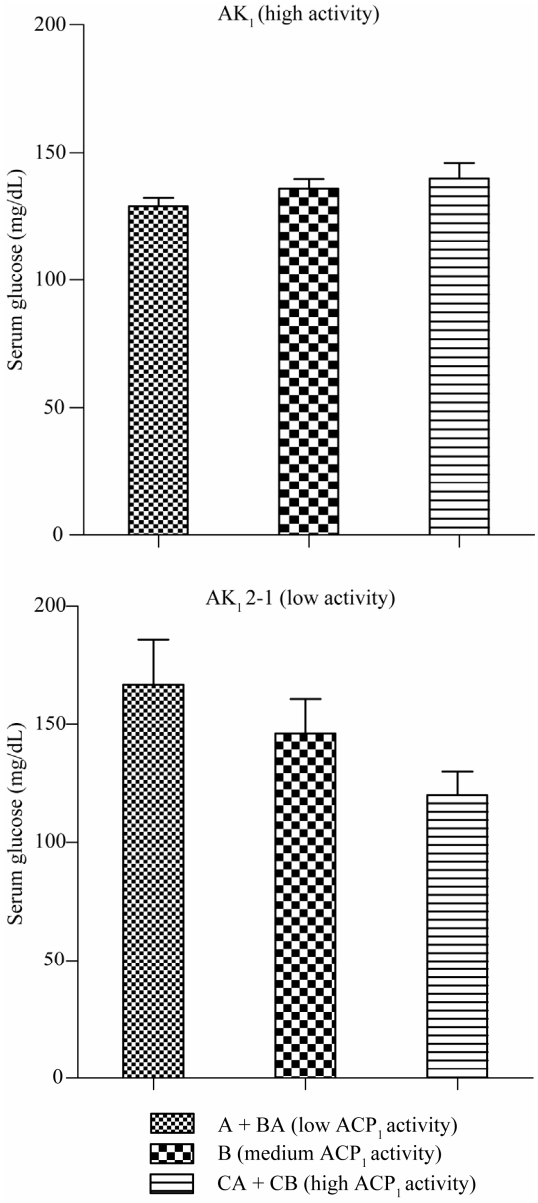

Figure 1. Blood glucose in T2D patients in relation to AK1 and ACP1 phenotypes. "AK_1-ACP_1 blood glucose interaction" ACP1 three classes ((A + BA) vs B vs (CA+CB)) "p = 0.079 ACP1 two classes ((A + BA) vs (C+CB))" p = 0.030.

ADP, AMP and adenosine could have negative effects on the clinical evolution of T2D.

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