

## A Study of Adenylate Kinase Locus 1 ( $Ak_1$ ) Genetic Polymorphism in Diabetic Pregnancy

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### Abstract

**Background:** Previous studies suggest that adenylate kinase locus 1 ( $Ak_1$ ) has an important role in the control of blood glucose level and in the glycation of structural and functional proteins in type 2 diabetes and in the balanced development of fetoplacental unit in healthy puerperae (HP). In this study, an attempt was made to investigate the relationship of  $Ak_1$  with maternal and neonatal parameters in puerperae with gestational diabetes (GDP) and with preexisting type 1 diabetes (T1DP).

**Methods:** This study was carried on 402 HP, 347 consecutive healthy newborns, 102 GDP and 111 T1DP with their newborn infants.  $Ak_1$  phenotype was determined by starch gel electrophoresis. Chi-square test of independence was carried out by SPSS program. The analysis of three way contingency table was carried out by a loglinear model. Significant level was 0.05.

**Results:** In T1DP, the frequency of  $Ak_1*2$  allele was higher than in GDP and in HP. Serum glucose level was higher in T1DP than in GDP with higher values in carriers of  $Ak_1*2$  allele. Neonatal hypoglycemia was more frequent in T1DP than in GDP with a positive association with  $Ak_1*2$  allele. The correlation between birth weight (BW) and placental weight (PW) was lower in infants from T1DP than HP. In healthy puerperae the correlation is higher in  $Ak_1$  2-1 than in  $Ak_1$ 1 phenotype while in diabetic puerperae the pattern is reversed with lower values in  $Ak_1$ 2-1 than in  $Ak_1$ 1 phenotype. The lowest value of correlation is observed in infants from T1D mothers carrying the  $Ak_1*2$  allele.

**Conclusion:** The data confirmed the involvement of  $Ak_1$  in glucose metabolism and showed a disturbance of the balance between placental and fetal growth which was more marked in T1DP.

**Keywords:** Adenylate kinase, BW-PW correlation, Gestational diabetes, Neonatal hypoglycemia, Preexisting T1D.

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### Introduction

Previous studies have shown an important role of adenylate kinase locus 1 ( $Ak_1$ ) genetic polymorphism in type 2 diabetes (T2D) (1, 2) and fetoplacental development (3, 4). In T2D,  $Ak_1$ 2-1 phenotype is associated to higher blood glucose levels and increased tendency to dyslipidemia and retinopathy. Moreover, the correlation between blood glucose and glycated hemoglobin is higher in  $Ak_1$ 2-1 than in  $Ak_1$ 1 phenotype

suggesting an important role of  $Ak_1$  genetic variation in the glycation of structural and functional proteins. In a large sample of healthy newborn infants, a higher correlation between birth weight (BW) and placental weight (PW) has been observed in infants with  $Ak_1$ 2-1 phenotype as compared with those with  $Ak_1$ 1 phenotype.

Adenylate kinase (AK) catalyzes the nucleotide phosphoryl interconversion of  $ATP+AMP \rightleftharpoons 2ADP$ .

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The products of this reaction are involved in the regulation of many cellular functions and relationships. Indeed, ATP represents a storage of energy for intracellular processes and for sending messages to nearby cells (5, 6).

The family of AK enzymes includes seven genes,  $Ak_1$ - $Ak_7$ , with different functions, molecular weight and kinetic property. The network of these enzymes regulates energetic and metabolic signaling circuits and fastens an efficient economy of cell energy, signal transduction and stress response (6). This family of enzymes removes phosphates from ATP, one by one, producing adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine that have different effects on cells by binding themselves to P2 (ADP and AMP) and to P1 (adenosine) receptors.

$Ak_1$  belongs to AK family loci and shows three phenotypes with different enzymatic activity, in the order  $Ak_11 > Ak_121 > Ak_12$  corresponding to the presence of two codominant alleles,  $Ak_1^*1$  and  $Ak_1^*$  on chromosome 9 (7).

In the present study, the relationship of  $Ak_1$  polymorphism with maternal and neonatal parameters was studied in women with gestational diabetes and in women with preexisting type 1 diabetes who had delivered a liveborn infant.

The aims of the present investigation were (i) to search for a possible relationship between  $Ak_1$  genetic variability and susceptibility to type 1 diabetes (T1D); (ii) to confirm the relationship between  $Ak_1$  phenotype and glycemic levels in puerperae with gestational diabetes (GDP) and in puerperae with type 1 diabetes (T1DP); (iii) to search for possible effect of  $Ak_1$  genetic variability on neonatal hypoglycemia; (iv) to study the effects of diabetes and  $Ak_1$  genetic variability on the correlation between placental and fetal growth.

### Methods

This study was carried out on 402 healthy puerperae (HP), 347 newborns from these mothers, 102 puerperae with gestational diabetes with their newborn infants and 111 puerperae with preexisting type 1 diabetes with their newborn infants. All samples were collected consecutively. All subjects were from the White population of Rome. The samples were collected in the Maternity Department of University Hospital. Blood samples were obtained from mothers by venipuncture. In newborns, the blood was collected from the placental side of umbilical cord after its section. Verbal informed consent was obtained from mothers

for participation in this study. The study was performed several years ago before the institution of an Ethical Committee and was approved by the Department of Obstetrics and Gynecology.

**Laboratory analysis:** Serum glucose concentration was measured by the automated Roche/Hitachi cobas C501 system based on enzymatic reaction with exokinase.

$Ak_1$  phenotype was determined by starch gel electrophoresis of haemolysate (7) as previously described (3). Samples were examined at pH=7. The inserts 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 containing glucose 10 mM, magnesium chloride 20 mM, adenosine diphosphate (ADP) 1 mM, nicotinamide 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 was incubated at 37°C for two hours.

At the sites of AK activity, ADP was converted into AMP and ATP. The ATP reacted with glucose in the presence of hexokinase to produce ADP and glucose-6-phosphate (G6P). This was oxidized to 6-phosphogluconate by G6PD with concomitant reduction of NADP. The reduced NADP in the presence of PMS caused the reduction of MTT to give a blue-coloured insoluble formazan, which was deposited at the sites of AK activity. In Caucasian populations, three distinct types of electrophoretic patterns were recognized referred to as  $Ak_11$ ,  $Ak_12-1$  and  $Ak_12$  corresponding to the presence of two codominant alleles,  $Ak_1^*1$  and  $Ak_1^*2$  at an autosomal locus.

**Statistical analysis:** Difference between proportions was evaluated by Chi-square test of independence. Bivariate correlation was evaluated by Spearman test. These analyses have been performed by SPSS program (Version 5.0, 1992, Chicago SPSS Inc.). The analysis of three way contingency table has been performed according to Sokal and Rohlf (8). By this test, it is possible to evaluate the effect of a third variable on the association between two variables. Significance level was fixed at 0.05.

Because of random missing values of variables studied, the number of subjects is not the same in all tables.

**Table 1.** *Ak<sub>1</sub>* phenotype and allele distribution in diabetic and healthy puerperae

Women sample	Proportion of <i>Ak<sub>1</sub></i> phenotypes			Total	Proportion of <i>Ak<sub>1</sub>*2</i> allele	Total
	<i>Ak<sub>1</sub></i>	<i>Ak<sub>1</sub>2-1</i>	<i>Ak<sub>1</sub>2</i>			
Gestational diabetes (a)	94.1%	5.9%	0.0%	101	2.97%	202
Preexisting type 1 diabetes (b)	89.2%	9.9%	1.00%	111	5.86% <sup>a</sup>	222
Healthy puerperae (c)	94.5%	5.5%	0.0%	402	2.74%	804

a: p=0.028, difference between PT1D and GD, HP

**Table 2.** Maternal glycaemic levels in relation to type of diabetes and *Ak<sub>1</sub>* phenotype

Serum glucose	Gestational diabetes		Preexisting T1D	
	Proportion of <i>Ak<sub>1</sub>*2</i> carriers	Total n	Proportion of <i>Ak<sub>1</sub>*2</i> carriers	Total n
115-140	6.0%	67	10.0%	41
140-165	15.4%	13	9.1%	33
>165	0.0%	3	18.0%	28

## Results

Table 1 shows maternal *Ak<sub>1</sub>* phenotype distribution in HP, GDP and T1DP. Phenotype and allele distribution were similar in HP and GDP. In T1DP, the proportion of *Ak<sub>1</sub>\*2* allele was higher as compared to HP and GDP (p<0.05).

Table 2 shows the level of maternal serum glucose in relation to *Ak<sub>1</sub>* phenotype and type of diabetes. The level of serum glucose was much higher in T1DP than in GDP. There was a tendency to higher serum glucose concentration in carriers of *Ak<sub>1</sub>\*2* but this effect was not statistically significant;

however, there was a significant additive effect of diabetes type with *Ak<sub>1</sub>* phenotype (p<0.001).

The relationship of neonatal hypoglycemia with the type of diabetes and neonatal *Ak<sub>1</sub>* phenotype was reported in table 3. Neonatal hypoglycemia was more frequent in T1DP than in GDP and the proportion of newborns with neonatal hypoglycemia was higher in newborns carrying the *Ak<sub>1</sub>\*2* allele. Although the effect of *Ak<sub>1</sub>* phenotype was not statistically significant, there was a significant additive effect of diabetes type and *Ak<sub>1</sub>* phenotype concerning their association with neonatal hypoglycemia (p<0.001). The same pattern was observed considering *Ak<sub>1</sub>* maternal phenotype (Table 4).

Table 5 shows the correlation between BW and PW in infants from HP, GDP and T1DP. Both neonatal and maternal *Ak<sub>1</sub>* phenotype have been considered. The correlation was generally lower in infant from diabetic puerperae as compared to newborns from healthy puerperae and this effect

**Table 3.** Neonatal hypoglycemia in relation to type of diabetes and neonatal *Ak<sub>1</sub>* phenotype

Neonatal hypoglycemia	Gestational diabetes		T1D	
	<i>Ak<sub>1</sub></i>	<i>Ak<sub>1</sub>2-1</i> and <i>Ak<sub>1</sub>2</i>	<i>Ak<sub>1</sub></i>	<i>Ak<sub>1</sub>2-1</i> and <i>Ak<sub>1</sub>2</i>
%Proportion of infants with neonatal hypoglycemia	8.8%	22.2%	33.7%	62.5%
Total n	91	9	101	8

**Table 4.** Neonatal hypoglycemia in relation to type of diabetes and maternal *Ak<sub>1</sub>* phenotype

Neonatal hypoglycemia	Gestational diabetes		T1D	
	<i>Ak<sub>1</sub></i>	<i>Ak<sub>1</sub>2-1</i> and <i>Ak<sub>1</sub>2</i>	<i>Ak<sub>1</sub></i>	<i>Ak<sub>1</sub>2-1</i> and <i>Ak<sub>1</sub>2</i>
%Proportion of infants with neonatal hypoglycemia	11.7%	0.00%	33.0%	58.3%
Total n	94	6	97	12

**Table 5.** Correlation (Spearman  $r_s$ ) between birth weight and placental weight according to newborn and maternal *Ak<sub>1</sub>* phenotypes

BW-PW Correlation	<i>Ak<sub>1</sub></i> newborn phenotype		<i>Ak<sub>1</sub></i> maternal phenotype	
	<i>Ak<sub>1</sub></i>	<i>Ak<sub>1</sub>2-1</i>	<i>Ak<sub>1</sub></i>	<i>Ak<sub>1</sub>2-1</i>
<b>Newborns from normal pregnancy</b>				
$r_s$	0.423	0.747	0.479	0.813
p	0.001	0.001	0.001	0.001
n	319	23	272	13
<b>Newborns from mothers with gestational diabetes</b>				
$r_s$	0.376	0.212	0.410	0.088
p	0.001	0.686	0.001	0.868
n	95	6	95	6
<b>Newborns from mothers with preexisting T1D</b>				
$r_s$	0.211	-0.064	0.396	-0.210
p	0.038	0.851	0.001	0.513
n	97	11	97	12

was more marked in T1DP than in GDP. While in infants from HP the correlation between BW and PW was higher in newborns and in mothers carrying *Ak<sub>1</sub>\*2* allele than in those carrying *Ak<sub>1</sub>* phenotype, in infants from diabetic puerperae the pattern was reversed and the correlation was lower in carriers of *Ak<sub>1</sub>\*2* allele than in infants and in mothers carrying *Ak<sub>1</sub>* phenotype. The lowest value of correlation coefficient was observed in infants from T1D mothers carrying *Ak<sub>1</sub>\*2* allele ( $p=0.003$  for the comparison of correlation coefficient between newborns from T1D mothers and newborns from healthy puerperae).

### Discussion

The present data confirmed the tendency in diabetic subjects to a higher serum glucose concentration in carriers of *Ak<sub>1</sub>\*2* allele as compared to subjects carrying *Ak<sub>1</sub>* phenotype (1, 2). T1DP showed a higher proportion of *Ak<sub>1</sub>\*2* allele as compared to HP and GDP suggesting that *Ak<sub>1</sub>\*2* allele may contribute to predisposition to T1D. This could be connected with the tendency to high glucose level in carriers of this allele. The susceptibility to neonatal hypoglycemia appeared to depend on both maternal diabetes and to the presence of *Ak<sub>1</sub>\*2* allele.

In nondiabetic mothers carrying the *Ak<sub>1</sub>\*2* allele, a slight increase of glycemic level associated to this phenotype may favor a harmonic growth of the two portions of the developing zygote. On the other hand, since *Ak<sub>1</sub>* 2-1 phenotype in diabetic mothers was associated to a high glycemic level and to a higher tendency to glycosylation of structural and functional proteins (1), this could contribute to the dissociation between birth weight and placental weight which was more marked in T1D.

Birth weight/placental weight development ratio was correlated with perinatal morbidity and mortality and with cardiovascular disease in adulthood (9, 10). More recent observations suggest an important role of placenta in fetal brain development and on neurologic and psychiatric outcomes in the child (11-13).

The limitation of the present study was represented by the relatively small number of subjects examined.

### Conclusion

*Ak<sub>1</sub>*2-1 phenotype in diabetic pregnant women may herald severe maternal hyperglycemia and neonatal hypoglycemia. Diabetic mothers with

this phenotype carried a risk of dissociation between birth weight and placental weight that could increase the susceptibility to perinatal morbidity and mortality and cardiovascular diseases in adulthood.

### Conflict of Interest

The authors declare no conflict of interest.

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