Short Communication

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 feto-placental 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.

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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 feto-placental development (3, 4). In T2D, $Ak_1$2-1 phenotype is associated to higher blood glucose levels and increased tendency to dyslipidemia and retinopatia. 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⇔2ADP.

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 signalling 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_1$1> $Ak_1$21> $Ak_1$2 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 pueperae 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*, 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 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_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.

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_1 phenotype and allele distribution in diabetic and healthy puerperae

Women sample	Proportion of Ak_1 phenotypes			Total	Proportion of Ak_1*2 allele	Total
	$Ak_1$1	$Ak_1$2-1	$Ak_1$2			
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% [a]	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 glycemic levels in relation to type of diabetes and Ak_1 phenotype

Serum glucose	Gestational diabetes		Preexisting T1D	
	Proportion of Ak_1*2 carriers	Total n	Proportion of Ak_1*2 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_1 phenotype distribution in HP, GDP and T1DP. Phenotype and allele distribution were similar in HP and GDP. In T1DP, the proportion of Ak_1*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_1 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_1*2 but this effect was not statistically significant; however, there was a significant additive effect of diabetes type with Ak_1 phenotype ($p< 0.001$).

The relationship of neonatal hypoglycemia with the type of diabetes and neonatal Ak_1 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_1*2 allele. Although the effect of Ak_1 phenotype was not statistically significant, there was a significant additive effect of diabetes type and Ak_1 phenotype concerning their association with neonatal hypoglycemia ($p<0.001$). The same pattern was observed considering Ak_1 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_1 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_1 phenotype

Neonatal hypoglycemia	Gestational diabetes		T1D	
	$Ak_1$1	$Ak_1$2-1 and $Ak_1$2	$Ak_1$1	$Ak_1$2-1 and $Ak_1$2
%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_1 phenotype

Neonatal hypoglycemia	Gestational diabetes		T1D	
	$Ak_1$1	$Ak_1$2-1 and $Ak_1$2	$Ak_1$1	$Ak_1$2-1 and $Ak_1$2
%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_1 phenotypes

BW-PW Correlation	Ak_1 newborn phenotype		Ak_1 maternal phenotype	
	$Ak_1$1	$Ak_1$2-1	$Ak_1$1	$Ak_1$2-1
Newborns from normal pregnancy				
r_s	0.423	0.747	0.479	0.813
p	0.001	0.001	0.001	0.001
n	319	23	272	13
Newborns from mothers with gestational diabetes				
r_s	0.376	0.212	0.410	0.088
p	0.001	0.686	0.001	0.868
n	95	6	95	6
Newborns from mothers with preexisting T1D				
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_1*2 allele than in those carrying $Ak_1$1 phenotype, in infants from diabetic puerperae the pattern was reversed and the correlation was lower in carriers of Ak_1*2 allele than in infants and in mothers carrying $Ak_1$1 phenotype. The lowest value of correlation coefficient was observed in infants from T1D mothers carrying Ak_1*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_1*2 allele as compared to subjects carrying $Ak_1$1 phenotype (1, 2). T1DP showed a higher proportion of Ak_1*2 allele as compared to HP and GDP suggesting that Ak_1*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_1*2 allele.

In nondiabetic mothers carrying the Ak_1*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_1 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_1$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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