PHOSPHOTYROSINE-PROTEIN-PHOSPHATASES AND HUMAN REPRODUCTION: AN ASSOCIATION BETWEEN LOW MOLECULAR WEIGHT ACID PHOSPHATASE (ACP1) AND SPONTANEOUS ABORTION

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SUMMARY

ACP1 (low molecular weight acid phosphatase) genetic polymorphism has been studied in 173 women with a history of two or more consecutive spontaneous abortions and in 1508 control subjects, including 482 normal pregnant women. The proportion of carriers of ACP1*C allele (*A/ *C, *B/*C) in women with a history of repeated spontaneous abortion is lower than in normal pregnant women and other control groups. Women with repeated spontaneous abortion show a specific decrease of ACP1 S isoform concentration as compared to normal pregnant women. The other component of ACP1 activity, the F isoform, does not show a significant difference between the two groups. The data suggest that women with ACP1 genotypes showing a high concentration of S isoform are relatively 'protected' against spontaneous abortion. Preliminary analysis of a sample of 352 normal puerperae along with their newborn babies supports this hypothesis.

KEY WORDS   ACP1   PTPases   Habitual abortion   Human reproduction

INTRODUCTION

Approximately 75% of embryos are lost during the early stages of intrauterine life (Diamond, 1987; Boklage, 1990). Both environmental and genetic factors are probably important determinants of such a high mortality, and it is likely that their effects are amplified by genomic instability during the early life stages (Vogel and Motulsky, 1986; Hicks, 1987). It is quite possible that common genetic polymorphisms play an important role in this process of intrauterine selection.

There has been a substantial interest recently in protein-tyrosine-phosphatases (PTPases) which may influence the action of growth factors through the regulation of phosphorylation state of critical target proteins (Goldstein, 1992; Fischer et al., 1991).

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One such PTPase is the highly polymorphic low molecular weight acid phosphatase (ACP1). In fact, recent experimental evidence indicates that ACP1 is able to dephosphorylate growth factor receptors (Stefani *et al.*, 1993). This enzyme is controlled by a locus on chromosome 2 having three common alleles: ACP1*A, ACP1*B and ACP1*C. There are quantitative differences in the enzymatic activity among ACP1 genotypes. Spencer *et al.* (1964) have found the following order of allelic contributions to the enzymatic activity: ACP1*A < ACP1*B < ACP1*C.

ACP1 is a member of a family of low molecular weight acid phosphatases that are found in human erythrocytes, in the rat liver and in other human and animal tissues. The animal enzymes have sequencies similar to the human ACP1 (Wo *et al.*, 1992; Camici *et al.*, 1989; Dissing and Svensmark, 1990; Dissing *et al.*, 1991; Manao *et al.*, 1992). Two important functions of ACP1 have been suggested based on the experimental evidence: flavin-phosphatase activity and tyrosine phosphatase activity (Wo *et al.*, 1992; Boivin and Galand, 1986; Mansfield and Sensabaugh 1978; Fuchs *et al.*, 1992). By catalysing the conversion of flavin-mononucleotide (FMN) to riboflavin, ACP1 may play a role in regulating the cellular concentration of flavin-adenin-dinucleotide (FAD), flavoenzyme activity and energy metabolism. As phosphotyrosine phosphatase (PTPase), the enzyme may affect the cellular growth regulation and the modulation of glycolytic rate by controlling receptor activities (Wo *et al.*, 1992; Boivin and Galand, 1986; Ramponi *et al.*, 1989; Ramponi *et al.*, 1992: Vogel *et al.*, 1993). Given that activity variants of ACP1 are common, it is possible that this enzyme is important in regulating a large spectrum of cellular functions.

Each allele of ACP1 locus encodes two isozymes, the fast (F) and the slow (S) that are expressed in an allele specific ratio: F/S=2:1, 4:1 and 1:4 for ACP1*A, *B and *C allele, respectively (Dissing, 1987). Significant differences between F and S isozymes have been observed in both enzymatic and molecular properties suggesting that F and S isozymes may serve different biological functions in the cell (Stefani *et al.*, 1993). On the other hand, the three genetically different F isozymes (Af, Bf, Cf) show identical properties as do the three S isozymes (Dissing, 1987). Thus, from a functional point of view, the common ACP1 phenotypes consist of only two different isozymes, F and S, the proportion of which determines the properties of the phenotypes.

Associations of ACP1 genotype with developmental parameters (Amante *et al.*, 1990), congenital malformations (Ward *et al.*, 1986) and fertility parameters (Chakraborty *et al.*, 1986) have been reported, all pointing to a significant role of ACP1 in human reproduction. In the current note, we present an analysis of data suggesting an association between ACP1 genotype and spontaneous abortion.

MATERIALS AND METHODS

Women with repeated spontaneous abortions

173 women who had at least two consecutive spontaneous abortions were examined. This condition is currently clinically classified as 'habitual abortion'. All the subjects were seen at the Center for Reproductive Disorders of the Department of Obstetrics and Gynecology at the 1st University of Rome. All couples wanted to have a child and requested medical assistance in general after the second or third episode of miscarriage but in some instances after the first episode. The following clinical examinations and tests are generally performed on a couple after two consecutive abortions: hysterosalpingo-

graphy, a study of the cycle with the registration of the basic temperature and the serial determination of the serum progesterone levels during the luteinic phase, glucose tolerance test, serological tests for lues and a test for Toxoplasma infection. At least three examinations of the seminal liquid are also performed. All of the clinical examinations and tests described above were consistently negative in the subjects included in the present sample. A standard karyotype analysis is also performed routinely in couples with habitual abortion.

Controls
Normal pregnant women

ACP1 genotypes were evaluated in three consecutive samples of women delivering a single live-born infant. Two of the samples were collected from the population of Rome, and one from the population of Penne, a small town in the Central-Eastern part of Italy. The distributions of ACP1 genotypes in the three samples were not significantly different, and the samples were combined into a single data set (Table 1).

Newborn infants

ACP1 genotype was determined in a consecutive sample of 608 newborn infants of both sexes from the population of Rome.

Normal adults

417 adults (both sexes) from the population of Rome previously studied by Modiano *et al.* (1967) were also considered as controls.

ACP1 genotype was determined according to Harris and Hopkinson (1976). F and S isoform concentrations were assigned to each genotype according to Dissing (1987).

Statistical analyses were carried out using SPSS programs (Nie *et al.*, 1975) on an IBM PC.

RESULTS

Table 1 shows the distribution of ACP1 genotypes in women with habitual abortions, in their husbands and in controls. The proportion of genotypes carrying the ACP1*C allele is lower in women with habitual abortion than in normal pregnant women, in husbands or in other normal controls. No statistically significant difference is observed between normal controls and normal pregnant women or husbands of women with habitual abortion. Reliable negative history of spontaneous abortion was obtained in 259 normal pregnant women. Compared with these subjects, women with habitual abortion again show a lower frequency of genotypes carrying the ACP1 *C allele. No significant difference is observed between women with two consecutive spontaneous abortions and women with three or more consecutive abortions. No significant association has been observed between ACP1 distribution and age in normal pregnant women. The proportion ACP1*C carriers is 12,8% in women less than 28 years old and 11, 96% in women more than 28 years old.

Table 2 shows the mean concentration of F and S ACP1 isoforms in women with habitual abortion and in normal pregnant women. A highly significant difference is observed in the S isoform concentration: the mean value is lower in women with habitual abortion than in normal pregnant women. On the other hand, the concentration of F isoform does not show a significant difference between the two samples.

Table 1. ACP1 distribution (per cent) in women with repeated spontaneous abortion and in control subjects.

	ACP1 genotypes							ACP1 alleles			
	*A/*A	*B/*B	*C/*C	*A/*B	*A/*C	*B/*C	total no.	*A	*B	*C	total no.
Women with repeated spontaneous abortions											
All (1)	10.4%	43.4%	0.0%	41.0%	0.6%	4.6%	173	31.2%	66.2%	2.6%	346
2 abortions (1a)	8.4%	49.4%	0.0%	37.4%	0.0%	4.8%	83	27.1%	70.5%	2.4%	166
3 or more abortions (1b)	12.2%	37.8%	0.0%	44.4%	1.1%	4.4%	90	35.0%	62.2%	2.8%	180
Husbands (2)	4.1%	49.1%	0.0%	33.1%	4.1%	9.5%	169	22.8%	70.4%	6.8%	338
Normal pregnant women											
All (3)	8.3%	40.7%	0.0%	38.4%	3.3%	9.3%	482	29.2%	64.5%	6.3%	964
With negative history of abortion (3a)	7.7%	39.0%	0.0%	41.3%	2.7%	9.3%	259	29.7%	64.3%	6.0%	518
Consecutive newborn babies (both sexes) (4)	9.5%	46.3%	0.5%	31.2%	3.1%	9.4%	609	26.7%	66.6%	6.7%	1218
Normal adults (both sexes) (5)	8.6%	43.9%	0.2%	31.6%	3.4%	12.2%	417	26.1%	65.8%	8.0%	834

Chi square test of independence

Comparisons	(*A/*A)vs(*B/*B)vs(*A/*B)vs(*C/*C+*A/*C+*B/*C)	(*C/*C+*A/*C+*B/*C)vs(other ACP1 genotypes)	(*C)vs(other ACP1 alleles)
(1)vs(2)vs(3)vs(4)vs(5)	p=0,0099	0,0144	0,0173
(4)vs(5)	p=0,5769	0,2308	0,3034
(1)vs(3)	p=0,0524	0,0099	0,0122
(1)vs(4+5)	p=0,0032	0,0018	0,0018
(2)vs(4+5)	p=0,1755	0,9507	0,8514
(3)vs(4+5)	p=0,0658	0,4849	0,3884
(1a)vs(1b)	p=0,4786	0,9007	0,9020
(1)vs(3a)	p=0,0911	0,0272	0,0312

Table 2. F and S ACP1 isoform concentration in women with repeated spontaneous abortion and in normal puerperae.

	F isoform		S isoform		no. of women
	mean	S.D.	mean	S.D.	
Women with repeated spontaneous abortion	13.43	2.9	4.08	1.9	173
Normal puerperae	13.23	2.9	4.72	2.9	482
Significance of difference (two tail)	p=0.451		p=0.002		

Table 3 shows the ratio of females carrying *C allele to females not carrying this allele in relation to the carrier status of their husbands. The proportion of *C carriers is much lower among women with a non carrier husband than among women with a carrier husband.

Table 4 shows the observed and expected distributions of ACP1 mating types (assuming control parameters and random mating). There is a strong reduction of mating types [female carrying *C allele] x [male not carrying *C allele] but not of mating types [female carrying *C allele] x [male carrying *C allele].

Preliminary data on ACP1 maternal-neonatal distribution in normal puerperae from the population of Penne are shown in Table 5. There is an excess of the type [mother carrying *C allele] x [offspring not carrying *C allele].

DISCUSSION

The term habitual abortion is well established in clinical practice, although there is a considerable disagreement about its definition, since the existence of a separate population of women with high-risk pregnancies has not been proven unambiguously yet. Selecting couples with repeated abortions in our study has a definite advantage of identifying spontaneous abortions. Indeed, while the correct assignment of a single episode of abortion as spontaneous or induced is generally difficult, all of our cases are certainly spontaneous. It is also likely that inherited factors may have a relatively greater importance in women with consecutive spontaneous abortions than in women with a single episode of miscarriage in whom stochastic factors may be more prevalent. Comparison of repeated with sporadic abortion (Table 1) shows that the association with ACP1 genotype is present only in habitual abortion thus supporting this conjecture.

The data in Table 1 indicates that women carrying ACP1*C allele which shows the highest ACP1 activity, are less likely to have a clinical recognizable spontaneous abortion, suggesting a 'protective' effect by this allele. It could be argued, however, that, since clinically recognizable spontaneous abortions represent only a small fraction (probably less than 10%) of the total loss of embryos during the intrauterine development (Chard, 1991), the 'protective' effect observed in women carrying an

Table 3. Ratio of female carriers of *C allele to female non carriers in relation to carrier status of husband.

		Husband	
		carrier of ACP1*C allele	non carrier
Wife	carrier of ACP1*C allele	5	4
	non carrier	18	142

per cent proportion of female carriers of *C allele	21.7%	2.7%

Fisher exact test   p<0.01

Table 4. ACP1 mating type distribution in couples with repeated spontaneous abortions. Expected values have been calculated on the basis of ACP1*C allele frequencies of normal controls (sample 5 of Table 1) and assuming Hardy-Weinberg equilibrium and random mating.

			Husband	
			not carrying ACP1*C	carrier
Wife	not carrying ACP1*C	observed	142	18
		expected	121.07	21.97
	carrier	observed	4	5
		expected	21.97	3.99

p<0.001

Table 5. ACP1 joint mother-infant type distribution in normal puerperae. Expected values have been calculated on the basis of mean value of ACP1*C allele frequencies of mothers and newborns and assuming Hardy-Weinberg equilibrium.

			Newborn	
			not carrying ACP1*C	carrier
Mother	not carrying ACP1*C	observed	283	19
		expected	289.4	19.5
	carrier	observed	30	20
		expected	19.5	23.6

comparison of carrier mothers bearing a non carrier newborn vs other types
p<0.02

ACP1*C allele might, in fact, be a result of an earlier loss of zygotes that is not clinically recognizable.

The data in Tables 3 and 4 demonstrate that the distribution of ACP1 genotypes among women with habitual abortions is dependent on the ACP1 genotype of their husbands suggesting that the 'mating type' of a 'couple' might be the most important determinant of susceptibility to spontaneous fetal loss. In fact, comparing the observed frequencies with those expected under the null hypothesis of independence, it is seen that the 'protective' effect of a maternal ACP1*C allele is present only if the husband does not carry such an allele. This suggests a preferential protection of foetuses not carrying ACP1*C. On the other hand, the analysis of the joint mother-infant distribution of ACP1 genotypes (Table 5) shows a statistically significant excess of *A/*A, *A/*B and *B/*B infants (i.e. not carrying the ACP1*C allele) born by mothers carrying an ACP1*C allele. Thus, these data on normal puerperae also support the hypothesis that foetuses not carrying ACP1*C have a relatively high probability of survival if the mother carries an ACP1*C allele.

It is likely that *C carrier mothers protect non-carrier zygotes during the early stages of development also. Only under such assumption, in fact, it is possible to explain the excess of newborns not carrying ACP1*C among normal puerperae carrying this allele.

Our study of habitual abortion started before 1980 and was interrupted for a short period in 1987. The association with ACP1 is very similar between the two samples: in the first series (68 couples) the proportion of women carrying ACP1*C allele is 5.9% and in the second series (105 couples) 4.8%.

Only S isoform is involved in the association with spontaneous abortion. This observation supports the conjecture that F and S isoforms may serve different biological functions.

The reproducibility of the pattern of association in samples collected in different times in the same population, the consistence of relevant aspects of assocation in different samples from diverse populations, and the specificity of association for only one ACP1 isoform make it very unlikely that the observed pattern may be due to mere sampling artefacts.

An association, however, does not represent the demonstration of a causal relationship. At present, the effect of other genes near ACP1 and in linkage disequilibrium with it cannot be excluded. However, the described association cannot be considered 'random'. In fact, we searched for it on the basis of 'a priori' knowledge of ACP1 functions which suggested relevance in intrauterine development, and we found a pattern of relationships consistent in independent samples and biologically plausible on the basis of the enzymatic properties of ACP1. Therefore, we are propense to consider ACP1 as causal in the association.

Protein-tryrosine phosphatases (PTPases), including ACP1, play an essential role in the control of a receptor signalling through the phosphotyrosine pathway. Since the phosphorylation state of critical target proteins is balanced by the action of kinases and phosphatases (Goldstein, 1992; Fischer et al., 1991; Hashimoto and Goldstein, 1992; Saad et al., 1992; Kahn and White, 1988), genetic variability of ACP1 enzymatic activity may influence the action of growth factors (Stefani et al., 1993), and, in turn, intrauterine development and survival. A modulation of the action of growth factors can be very critical in the early stages of development, when the genomic stability is probably reduced. The association of ACP1 genotype with spontaneous abortion as well as the

associations with congenital fetal malformations (Ward *et al.*, 1986) and intrauterine growth (Amante *et al.*, 1990) support this hypothesis.
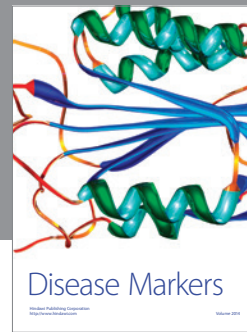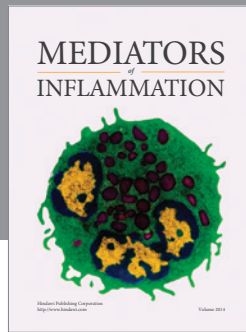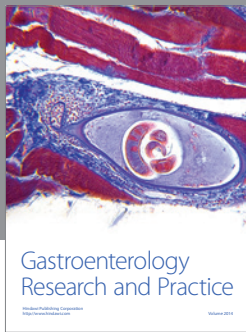
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