The genetics of feto-placental development: A study of acid phosphatase locus 1 and adenosine deaminase polymorphisms in a consecutive series of newborn infants

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Abstract

Background: Acid phosphatase locus 1 and adenosine deaminase locus 1 polymorphisms show cooperative effects on glucose metabolism and immunological functions. The recent observation of cooperation between the two systems on susceptibility to repeated spontaneous miscarriage prompted us to search for possible interactional effects between these genes and the correlation between birth weight and placental weight. Deviation from a balanced development of the feto-placental unit has been found to be associated with perinatal morbidity and mortality and with cardiovascular diseases in adulthood.

Methods: We examined 400 consecutive newborns from the Caucasian population of Rome. Birth weight, placental weight, and gestational length were registered. Acid phosphatase locus 1 and adenosine deaminase locus 1 phenotypes were determined by starch gel electrophoresis and correlation analysis was performed by SPSS programs. Informed verbal consent to participate in the study was obtained from the mothers.

Results: Highly significant differences in birth weight-placental weight correlations were observed among acid phosphatase locus 1 phenotypes (p = 0.005). The correlation between birth weight and placental weight was markedly elevated in subjects carrying acid phosphatase locus 1 phenotypes with medium-low F isoform concentration (A, CA and CB phenotypes) compared to those carrying acid phosphatase locus 1 phenotypes with medium-high F isoform concentration (BA and B phenotypes) (p = 0.002). Environmental and developmental variables were found to exert a significant effect on birth weight-placental weight correlation in subjects with medium-high F isoform concentrations, but only a marginal effect was observed in those with medium-low F isoform concentrations. The correlation between birth weight and placental weight is higher among carriers of the adenosine deaminase locus 1 allele*2, which is associated with low activity, than in homozygous adenosine deaminase locus 1 phenotype 1 carriers (p = 0.04). The two systems show a cooperative effect on the correlation between birth weight and placental weight: the highest value is observed in newborns carrying adenosine deaminase locus 1 allele*2 and acid phosphatase locus 1 phenotypes with medium-low F isoform concentration (p = 0.005).

Conclusion: These data suggest that zygotes with low adenosine deaminase locus 1 activity and low F activity may experience the most favourable intrauterine conditions for a balanced development of the feto-placental unit.
Background
We have recently described a cooperative interaction between ACP1 (acid phosphatase locus 1) and ADA1 (adenosine deaminase locus 1) genetic polymorphisms concerning their effects on the susceptibility to spontaneous primary repeated miscarriages: women carrying the ADA1*2 and ACP1*C alleles show the lowest susceptibility to repeated miscarriages [1]. Both systems share important effects on glucose metabolism and immunological function. These observations prompted us to search for a possible cooperative interaction between the two systems regarding their effects on developmental parameters during intrauterine life.

It is likely that a balanced growth of the two portions of the feto-placental unit (i.e. without the prevalence of placental on the fetal part or vice versa) represents an advantage for fetal development. The birth weight/placental weight ratio (BW/PW) has been found to be correlated with perinatal morbidity and mortality and with cardiovascular disease in adulthood [2,3]. There is evidence that in addition to maternal factors and socioeconomic status, genetic factors also influence the ratio BW/PW [3-5]. In a recent note we have proposed the correlation between BW and PW as an index of balanced development of the feto-placental unit and have shown that this correlation is influenced by ACP1 phenotype [6].

The ACP1 genetic polymorphism
ACP1, also called low molecular weight phospho-protein tyrosine phosphatase (LMPTP) is an enzyme controlled by a locus on chromosome 2 showing three common alleles: ACP1*A, ACP1*B, ACP1*C. These three alleles are associated with different enzymatic activities [7]. Activity of ACP1 phenotypes are in the following order: A < BA < B = CA < CB < C [8].

Each allele at the ACP1 locus encodes two isoforms, called F (fast) and S (slow) [9,10]. ACP1 B and BA show a medium-high F isoform activity while CB, A, CA and C phenotypes show a medium-low F isoform activity. ACP1 C, CA and CB show a much higher activity of S isoform as compared to other ACP1 phenotypes [7,10].

Two important functions have been suggested for ACP1: flavin-mono-nucleotide phosphatase activity and tyrosine phosphatase activity [11-13]. Catalysing the conversion of flavin-mono nucleotide (FMN) to riboflavin, ACP1 may have a role in regulating the cellular concentration of flavin-adenine-dinucleotide (FAD), flavo-enzyme activity and energy metabolism. As a phosphotyrosine phosphatase, the enzyme may have an important role in cellular growth regulation and in modulation of glycolytic rate through the control of receptor activities and of band 3 protein phosphorylation status [[12,14] and [15]].

Recently it has been shown that ACP1 specifically dephosphorylates the negative regulatory Tyr-292 of ZAP-70, thereby counteracting inactivation of ZAP-70. The ZAP-70 protein-tyrosine kinase plays a central role in signalling from the T cell receptor. Thus, these results indicate that ACP1 strengthens T cell receptor signalling [16].

The ADA1 genetic polymorphism
ADA1 is a polymorphic enzyme present in all mammalian tissues [17]. It is controlled by a locus with two codominant alleles ADA*1 and ADA*2 located on the long arm of chromosome 20. The corresponding three common ADA1 phenotypes have different enzymatic activities: the ADA11 phenotype is 15% more active than the ADA12/1 phenotype and 30% more active than the ADA12 phenotype, which is very rare [18].

ADA1 catalyses the irreversible deamination of adenosine to inosine. Red Blood Cells (RBC) are in equilibrium with freely diffusing adenosine [19], pointing to an important role for this enzyme in the regulation of adenosine concentration.

Current interest has been focused on a wide variety of effects produced by adenosine via activation of cell surface adenosine receptors [20,21]. Adenosine counteracts insulin action in the liver by activating A2B receptors [22]. Adenosine seems to facilitate insulin action in adipocytes.

The adenosine deaminase complex protein [23] (ADPC) is identical with CD26, a T cell activating antigen and with a glycoprotein present in epithelial cells of various tissues. Recent data suggest that ADA1 and CD26 are co-localized on the T cell surface but not inside cells.

Cells expressing ADA1 and CD26 on the surface are much more resistant to the inhibitory effects of adenosine. These data suggest that ADA1 on the cell surface is involved in an important immunoregulatory mechanism by which released ADA1 binds to the cell surface of CD26, and this complex is capable of reducing the local concentration of adenosine [24].

In the present paper we have performed a more detailed analysis of the effect of ACP1 polymorphism and have extended this study to the ADA1 polymorphism. On the basis of the observations on women with repeated spontaneous miscarriages we would expect an optimal developmental context in zygotes carrying the ADA1*2 and ACP1*C alleles.

Methods
In the present study we examined 400 consecutive newborn infants from healthy puerperae. All infants were Caucasian from the population of Rome. Birth weight and
placental weight (wet, untrimmed) were registered in the delivery room. Gestational length was estimated from the date of the last menstrual period and checked against Dubowitz score as an additional index of neonatal maturity. Multiples were excluded. Mode of delivery does not alter the phenotype of enzyme considered.

The data presented in the paper were collected a few years ago and at that time there was not an established Ethical Committee. The project was discussed and approved in the Department. Informed verbal consent to participate in the study was obtained from the mothers. This has been recently (April 28, 2008) approved by the Institutional Ethical Committee.

Newborn blood samples were obtained from the placental side of the umbilical vein after umbilical cord section. The ACP1 phenotype was determined in 361 newborns by starch gel electrophoresis on red blood cell hemolysates according to Harris and Hopkinson [25]. The acid phosphatase pattern is revealed by a solution of phenolphthalein diphosphate: the addition of ammonium solution reveals the area where phenolphthalein has been liberated in the areas of gel where ACP1 activity is present. In European populations the presence of three common alleles *A, *B and *C determines the occurrence of six phenotypes: A, AB, B, AC, BC and C. Each of the homozygous A, B and C phenotypes are composed of two fractions, F and S, corresponding to fast and slow components of the electrophoretic pattern. Heterozygous phenotypes have a pattern corresponding to a mixture of homozygous types.

The ADA1 phenotype was determined by starch gel electrophoresis on red blood cell hemolysates according to Spencer et al [26]. Inosine produced at the sites of ADA1 isozymes and their relative intensities and relative electrophoretic mobilities. In the ADA12 type there are also three regularly spaced components which exhibit decreased staining intensity in order of their anodal electrophoretic mobility. Multiples were excluded. Mode of delivery does not alter the phenotype of enzyme considered.

The ADA12 pattern is appreciably slower than the ADA 11 pattern. The pattern exhibiting four isozymes, designated ADA2/1, has the appearance of a mixture of ADA1 and ADA2 patterns.

In the last few years, in our laboratory, determination of ADA1 and ACP1 genotypes has been performed routinely on DNA. In our laboratory the comparison of classical with DNA methods has shown practically no differences between phenotypic and genotypic classifications. On a sample of 50 subjects in which ACP1 and ADA1 phenotypes were determined by DNA and classical methods only one difference was observed for ACP1 and no difference for ADA1.

Correlation analysis was performed by SPSS programs. Differences between correlation coefficients were evaluated according to Snedecor and Cochran [27]. The distribution of ACP1 phenotypes among newborns does not differ statistically from Hardy-Weinberg expectation.

**Results**

Table 1 shows demographic parameters of the sample study. Table 2 shows the distribution of ACP1 phenotypes and developmental parameters for each phenotype. No statistical significant difference among ACP1 phenotypes is observed for BW, PW and gestational duration. CA phenotype shows low values for all parameters, but these values are not statistically different from those of other ACP1 phenotypes. BW-PW correlation analysis shows highly significant differences among ACP1 phenotypes. The highest correlation coefficient is observed for CA phenotype and the lowest for B phenotype.

Figures 1, 2, 3, and 4 illustrate the relationship between BW-PW correlation and relevant ACP1 parameters: F and S activity, F/S activity ratio and total activity. The BW-PW correlation is negatively associated with F concentration (Fig 1) and F/S ratio (Fig 3). A, CA and CB phenotypes that share a medium-low F activity and F/S ratio have a high BW-PW correlation, while the B phenotype, which has the highest F activity and F/S ratio, has a low BW-PW correlation. No association is observed for S isofrom concentration (Fig 2) and ACP1 total activity (Fig 4).

Table 3 shows the effect of smoking, maternal age, gestational age, parity and gender on the relationship between ACP1 and BW-PW correlation. For this analysis two classes of ACP1 phenotypes have been considered: A, CA, CB phenotypes with medium-low F activity, and B and BA phenotypes with medium-high F activity. In A, CA, CB

| Table 1: Maternal and neonatal parameters in the sample study |
|---------------------------------------------------------------|
| Mean | Proportion | S.E. |
|---------------------------------------------------------------|
| Maternal Age (yrs) | 28.5 | 0.3 |
| Gestational Age (wks) | 39.6 | 0.12 |
| Birth Weight (gr) | 3269 | 29 |
| Placental Weight (gr) | 578 | 8 |
| Smokers | 38% |
| Male Infant | 54% |
phenotypes only maternal age exhibits some effect on the BW-PW correlation, while among B and BA phenotypes most variables show highly significant effects on the BW-PW correlation.

Table 4 shows the distribution of ADA1 phenotypes and developmental parameters for the ADA11 phenotype and for carriers of the ADA1*2 allele. The BW-PW correlation coefficient is higher among carriers of the ADA1*2 allele than among ADA11 phenotype carriers (p = 0.04). No significant difference between ADA1 phenotypes has been observed for BW, PW and gestational duration.

Table 5 shows the effect of smoking, maternal age, gestational age, parity and gender on the relationship between ADA1 and BW-PW correlation. Significant effects of these variables were observed in the correlations of BW-PW in both ADA1 subjects and in carriers of the ADA1*2 allele.

The BW-PW correlation is influenced by environmental and developmental variables, but as shown in tables 3 and 5 the differential effects of ACP1 and ADA1 phenotypes on this correlation do not appear in general to be significantly influenced by these variables. Table 6 shows the correlation between BW and PW according to the joint ACP1-ADA1 phenotype. Both ADA1 and ACP1 phenotypes were determined in 327 infants. There is a highly significant difference among joint phenotypes (p = 0.000): the highest correlation is observed in subjects who carry A or CA or CB phenotypes and the ADA1*2 allele, while the lowest correlation is observed when the ADA1 1 phenotype is associated with B or BA phenotypes.

**Discussion and conclusion**

The present data suggest that foetuses with low ADA1 activity, associated with medium-low ACP1 F isoform activity, have the best correlation between BW and PW, suggesting a most favourable situation for the development of the feto-placental unit. These observations agree with those expected on the basis of previous data on women with repeated spontaneous miscarriages that demonstrated a cooperative protective effect of ADA1*2 and ACP1*C alleles against fetal loss. Thus, the two lines of evidence support the hypothesis that foetuses with low ADA1 activity and low ACP1 F isoform activity have a balanced development of feto-placental unit and a higher probability of survival compared to other foetuses.
**The relationship between F isoform concentration and BW-PW correlation.** The term BW-PW correlation expresses the correlation between birth weight and placental weight. ACP1 is the acid phosphatase locus 1. A, B, C, BA, CA, CB are the ACP1 phenotypes. In abscissa F isoform concentrations of each ACP1 phenotype are also reported. The rank correlation coefficient according to Spearman (27) between BW-PW correlation and F isoform concentration is $r_s = -1$, $p < 0.01$.

**The relationship between S isoform concentration and BW-PW correlation.** The term BW-PW correlation expresses the correlation between birth weight and placental weight. ACP1 is the acid phosphatase locus 1. A, B, C, BA, CA, CB are the ACP1 phenotypes. In abscissa S isoform concentrations of each ACP1 phenotype are also reported. The rank correlation coefficient according to Spearman (27) between BW-PW correlation and S isoform concentration is $r_s = 0.3$, $p$ not significant.

**The relationship between F/S isoform concentration and BW-PW correlation.** The term BW-PW correlation expresses the correlation between birth weight and placental weight. ACP1 is the acid phosphatase locus 1. A, B, C, BA, CA, CB are the ACP1 phenotypes. In abscissa F/S isoform concentrations of each ACP1 phenotype are also reported. The rank correlation coefficient according to Spearman (27) between BW-PW correlation and F/S isoform concentration is $r_s = -0.9$, $p < 0.05$.

**The relationship between F+S isoform concentration and BW-PW correlation.** The term BW-PW correlation expresses the correlation between birth weight and placental weight. ACP1 is the acid phosphatase locus 1. A, B, C, BA, CA, CB are the ACP1 phenotypes. In abscissa F+S isoform concentrations of each ACP1 phenotype are also reported. The rank correlation coefficient according to Spearman (27) between BW-PW correlation and F+S isoform concentration is $r_s = 0.3$, $p$ not significant.
The exact mechanism underlying the statistical association of ADA1 and ACP1 with the BW-PW correlation is not known at present. An immunological mechanism is supported by the well known relationship between ADA1 and immune diseases and between ACP1 and T cell activation. A relative depression of T cell activation due to low level of F ACP1 isoform and to higher concentration of adenosine (due to the low activity of ADA1*2 carriers) could modulate the feto-maternal immunological relationship resulting in a balanced development of the two portions of feto-placental unit.

A metabolic mechanism may be operative in which ACP1, acting as phosphotyrosine phosphatase, could have an important role in the modulation of glycolytic rate through the control of insulin receptor activity and of band 3 protein phosphorylation status. Additionally, catalysing the conversion of flavin-mononucleotide (FMN) in riboflavin, the enzyme may influence flavo-enzyme activity and energy metabolism [7]. In turn, with respect to ADA1 activity, recent studies have shown that adenosine counteracts insulin action in the liver by activating A2B receptors [20-22]. On the basis of these actions on glucose metabolism, ACP1 F isoform activity coupled with low ADA1 activity could have favourable effects on the development of the feto-placental unit.

Table 3: Correlation between birth weight and placental weight in relation to ACP1 F isoform activity.

| Sample                      | Medium-high (B+BA) | Medium-low (A+CA+CB) |
|-----------------------------|--------------------|-----------------------|
|                             | r | p* | r | p* |
| All subjects                | 0.209 | 0.001 | 0.687 | 0.000 |
| Smoking                     | | | | |
| Yes                         | 0.122 | 0.243 | 0.696 | 0.000 |
| No                          | 0.241 | 0.002 | 0.710 | 0.000 |
| Significance of difference  | 0.000 | 0.750 |
| Maternal age (yrs)          | | | | |
| ≤ 28 (28)                   | 0.027 | 0.745 | 0.723 | 0.000 |
| > 28 (28)                   | 0.478 | 0.000 | 0.640 | 0.000 |
| Significance of difference  | 0.000 | 0.025 |
| Gestational age (wks)       | | | | |
| ≤ 37 (37)                   | 0.183 | 0.440 | 0.731 | 0.160 |
| > 37 (37)                   | 0.228 | 0.000 | 0.655 | 0.000 |
| Significance of difference  | 0.450 | 0.950 |
| Birth order                 | | | | |
| 1                           | 0.305 | 0.001 | 0.672 | 0.000 |
| ≥ 2 (2)                     | 0.158 | 0.060 | 0.703 | 0.000 |
| Significance of difference  | 0.000 | 0.350 |
| Sex                         | | | | |
| Male                        | 0.171 | 0.044 | 0.712 | 0.000 |
| Female                      | 0.301 | 0.001 | 0.655 | 0.000 |
| Significance of difference  | 0.000 | 0.170 |

Significance of difference between correlation coefficients has been calculated according to Snedecor and Cochran. p* refers to significance of correlation coefficient. p** refers to significance of difference between the two classes. For “All subjects” the difference of correlation coefficients between (B+BA) vs (A+CA+CB) i.e. 0.209 vs 0.687 is highly significant: p = 0.002.
Regarding the effect of genetic variability of ACP1 on the correlation of BW with PW, it is interesting to speculate on the possible selective advantage of the *A and *C alleles over the *B allele. The *B allele is the most frequent in all human populations, and the *A allele is present with variable frequencies in all major ethnic groups, while *C allele is present with appreciable frequencies only in Caucasians.

Our data suggest that the optimal BW-PW correlation is seen in carriers of the ACP1*C allele and in the homozygous A phenotype (table 4 and Fig 1), while the heterozygous BA phenotype shows an intermediate value between B and A (Fig 1). Interestingly, in A, CA and CB phenotypes the BW-PW correlation is hardly influenced by environmental circumstances, while in B and BA phenotypes the environmental variables exert considerable effects on this correlation. Thus, ACP1*A and *C variants could have a selective advantage during intrauterine life on the fundamental ACP1*B allele. This might have contributed to an increase in frequencies of the ACP1*A and *C alleles to polymorphic values and could presently contribute to maintenance of the ACP1 polymorphism in human populations.

### Competing interests
The authors declare that they have no competing interests.

### Table 4: Parameters distribution of newborns in relation to ADA1 phenotypes.

| ADA1 phenotypes | Significance of difference between phenotypes (p) |
|-----------------|--------------------------------------------------|
| ADA1A           | (ADA12/1+ADA12)                                  |
| Absolute frequencies | 317 58                            |
| Percent frequencies | 84.5% 15.5%                      |

### Birth weight (gr)

|          | ADA1A | ADA1B |
|----------|-------|-------|
| Mean     | 3279  | 3311  |
| S.E.     | 32    | 54    |

### Placental weight (gr)

|          | ADA1A | ADA1B |
|----------|-------|-------|
| Mean     | 584   | 545   |
| S.E.     | 10    | 13    |

### Gestational age (wks)

|          | ADA1A | ADA1B |
|----------|-------|-------|
| Mean     | 39.63 | 39.84 |
| S.E.     | 0.13  | 0.24  |
| Median   | 40.00 | 40.00 |

### Correlation between birth weight and placental weight (r)

|          | ADA1A | ADA1B |
|----------|-------|-------|
| Mean     | 0.289 | 0.552 |
| S.E.     | 0.000 | 0.000 |

|          | ADA1A | ADA1B |
|----------|-------|-------|
| Significance of r (p) | 0.040** |

Significance of difference between means (*) refers to Variance analysis. Significance of difference between correlation coefficients (**) has been calculated according to Snedecor and Cochran.
Table 5: Correlation between birth weight and placental weight in relation to ADA1 phenotypes.

| Sample | ADA1 | (ADA2/1+ADA2) |
|--------|-----------------|-----------------|
|        | r    | p*  | r    | p*  |
| All subjects | 0.289 | 0.000 | 0.552 | 0.000 |

Smoking

| Smoking | r    | p*  | r    | p*  |
|---------|-----------------|-----------------|
| Yes  | 0.275 | 0.003 | 0.484 | 0.036 |
| No   | 0.288 | 0.000 | 0.589 | 0.000 |

Significance of difference p** 0.300 0.150

Maternal age (yrs)

| Maternal age (yrs) | r    | p*  | r    | p*  |
|-------------------|-----------------|-----------------|
| ≤28   | 0.104 | 0.181 | 0.679 | 0.000 |
| >28   | 0.559 | 0.000 | 0.341 | 0.120 |

Significance of difference p** 0.000 0.000

Gestational age (wks)

| Gestational age (wks) | r    | p*  | r    | p*  |
|----------------------|-----------------|-----------------|
| ≤37     | 0.292 | 0.187 | 0.922 | 0.078 |
| >37     | 0.286 | 0.000 | 0.560 | 0.000 |

Significance of difference p** 0.900 0.350

Birth order

| Birth order | r    | p*  | r    | p*  |
|-------------|-----------------|-----------------|
| 1           | 0.449 | 0.000 | 0.572 | 0.001 |
| ≥ 2         | 0.212 | 0.009 | 0.543 | 0.003 |

Significance of difference p** 0.000 0.700

Sex

| Sex | r    | p*  | r    | p*  |
|-----|-----------------|-----------------|
| male | 0.268 | 0.000 | 0.359 | 0.078 |
| female | 0.344 | 0.000 | 0.692 | 0.000 |

Significance of difference p** 0.000 0.000

Significance of difference between correlation coefficients has been calculated according to Snedecor and Cochran. p* refers to significance of correlation coefficient. p** refers to significance of difference between the two classes. For "All subjects" the difference of correlation coefficients between ADA1 and (ADA2/1+ADA2) i.e. 0.289 vs 0.552 is statistically significant: p = 0.04.

Table 6: Correlation between birth weight (BW) and placental weight (PW) according to the joint ACP1-ADA1 phenotype.

| Joint ACP1-ADA1 phenotype | Presence of ADA*2 allele | Presence of A or CA or CB types | BW-PW correlation coefficients (r) | Significance of r (p) | Total n° |
|---------------------------|--------------------------|---------------------------------|-----------------------------------|------------------------|----------|
|                           | -                        | -                               | 0.193                             | 0.000                  | 217      |

Significance of difference among correlation coefficients p = 0.000. Significance of difference among correlation coefficients has been calculated according to Snedecor and Cochran.

Authors’ contributions

GBF, BE, MA and BA have been involved in the conception and design of the study, in drafting the manuscript and in its critical revision. GBF and BE have interpreted the data and performed the statistical analyses. PA and CL have contributed to the revision of the manuscript, to the acquisition of the data and to coordination of study. All authors have read and approved the final manuscript.

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