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ACP_1 – ADA_1 Interaction and Human Health

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Abstract:

Background: Biochemical and epidemiological evidence suggest that ACP_1 and ADA_1 genetic polymorphisms interact with significant phenotypic effects at the clinical level. We have reviewed the observations collected by our group over twenty years on the interaction between these polymorphisms in order to elucidate their impact on human health.

Methods: ACP_1 and ADA_1 phenotypes from 3297 subjects were determined by starch gel electrophoresis on RBC hemolysates.

Results: Birth weights are higher in newborns with the ACP_1*A/*A and *B/*A genotypes carrying the ADA_1*2 than in those with other joint genotypes. During the pre-reproductive and early reproductive stages of extrauterine life, individuals carrying these genotypes are more susceptible to diseases involving the immune system. ACP_1*A/*A and *B/*A genotypes carrying the ADA_1*2 allele are protected against common diseases occurring during the last stage of life.

Conclusions: The joint ACP_1-ADA_1 system has an important role in human health and development.

Keywords: ACP_1; ADA_1; human health; genetic interaction

Introduction

Biochemical and epidemiological evidence suggest that Acid Phosphatase locus 1 (ACP_1) and Adenosine Deaminase locus 1 (ADA_1) genetic polymorphisms interact with significant phenotypic effects at the clinical level . In the present paper we review the observations collected by our group in the last twenty years in order to elucidate their roles on human health [1-7].

Genetic polymorphism of ACP_1

ACP_1 is a polymorphic enzyme controlled by a gene on chromosome 2. In European population there are 3 common codominant alleles: ACP_1*A, ACP_1*B and ACP_1*C. ACP_1 functions as a phosphatase and these three distinct alleles exhibit markedly different enzymatic activities. The total enzymatic activity for the six ACP_1 phenotypes measured with p-nitrophenylphosphate as a substrate, is in the order *A/*A<*A/*B<(*B/*B≤*A/*C)<*B/*C<*C/*C.

Two biological substrates have been suggested for ACP1: flavin-mononucleotide (FMN) and phosphorylated protein-tyrosine residues. Catalysing the conversion of FMN to riboflavin, ACP1 may regulate the cellular concentration of Flavin Adenine Dinucleotide (FAD),flavo-enzyme activity and energy metabolism. As a protein phosphotyrosine phosphatase,ACP_1 may have an important role in modulating the glycolitic rate through the control of insulin receptor activity and of band 3 protein phosphorylation status [8].

ACP_1 dephosphorylates a negative phosphorylation site in Zeta-chain-associated protein kinase 70 (ZAP 70) in T cells [9]. This event leads to increased activation of the kinase and enhanced signalling from T cell antigen receptor.

ACP_1 negatively modulates insulin signal transduction [10] and is able to dephosphorylate the Adipocyte Lipid Binding Protein (ALBP) in vitro [11]. ALBP belongs to a family of lipid binding proteins present in various isoforms in many human tissues. In adipose tissue ALBP is phosphorylated on Tyr19 after insulin stimulation thereby impairing its fatty acid binding ability . In the adipose tissue the dual activities of ACP_1 (on insulin receptor transduction and ALBP dephosphorylation) could partly compensate for each other.

Genetic polymorphism of ADA_1

ADA_1 is a polymorphic enzyme present in all mammalian tissues . It is controlled by a gene with two codominant alleles ADA_1*1 and ADA_1*2 located on the long arm of chromosome 20. Similar to ACP_1 the polymorphism of this enzyme exhibits different enzymatic activities between phenotypes in the order ADA_1*1/*1>ADA_1*2/*1>ADA_1*2/*2. ADA_1 catalyses the irreversible deamination of adenosine to inosine thereby contributing to the regulation of intracellular and extracellular concentration of adenosine. Experimental studies have shown that adenosine, acting via the A1R, increases insulin sensitivity in isolated adipocytes [12-14] and

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decreases insulin sensitivity in isolated muscle fibres [15,16].

ADA was thought to be an exclusively cytosolic enzyme until the recent demonstration that it is present on the surface of erythrocytes and platelets . Subsequently, it has been demonstrated that ADA is present on the surface of many cell types including lymphocytes and neurons, where it can act as an ectoenzyme [17,18]. The functional role of ecto-ADA is related not only to degradation of extracellular adenosine [19], but also to transduction signals through its interaction with either CD26 or Adenosine 1 Receptor (A1R), two ADA anchoring proteins. In this manner ADA acting as a costimulatory molecule integrates multiple signalling events in different cell types [20]. Adenosine, a purine nucleoside present in plasma and other extracellular fluids is an important local hormone (like prostaglandins) that regulates blood flow, neurotransmission, physiology of smooth muscle and platelet aggregation. Current interest has been focused on a wide variety of effects produced by adenosine via activation of cell surface adenosine receptors [21,22]. Recent studies indicates that adenosine counteracts insulin action in the liver by activating A2B receptors [23] while also facilitating insulin action in adipocytes.

Adenosine Deaminase Complex Protein (ADPC) is identical with CD26 [24], a T cell activating antigen and with a glycoprotein present in epithelial cells of various tissues. Recent data suggest that ADA_1 and CD26 are co-localized on the T cell surface but not inside cells. Cells expressing ADA_1 and CD26 on the surface are much more resistant to the inhibitory effects of adenosine. These data suggest that ADA_1 on the cell surface is involved in an important immunoregulatory mechanism by which released ADA_1 binds to the cell surface of CD26, and this complex is capable of reducing the local concentration of adenosine [19].

<u>The interaction between ACP_1 and ADA_1</u>

ACP_1 is modulated by various substances including purines [25] and its activity is influenced by ADA_1 genotype: in carriers of ADA_1*2 allele the activity is generally lower than in homozygotes for the ADA_1*1 allele [2]. There is also an interaction between ADA_1 and ACP_1 genotypes concerning their effects on ACP_1 activity. ACP_1*A/*A carrying the ADA_1*2 allele shows a 37% lower activity than ACP_1*A/*A with the ADA_1*1/*1 genotype; ACP_1*A/*B carrying the ADA*2 allele shows a 13% lower activity than ACP_1*A/*B with the ADA_1*1/*1 type, whereas for other ACP_1 types no significant differences have been observed between carriers of ADA_1*2 and subjects with ADA_1*1/*1 genotype. As a result, a very low ACP_1 activity is observed in individuals having ACP_1*A/*A genotype and carrying ADA_1*2 allele. Overall in *A/*A and *B/*A phenotypes carrying the ADA_1*2 allele there is a mean decrease of 18.5% of ACP_1 enzymatic activity. Therefore, effects related to low ACP_1 activity (i.e. *A/*A and *B/*A genotypes) could be modified in individuals carrying the ADA_1*2 allele.

Adenosine and inosine show modulating effects on ACP_1. The activation by inosine is much higher than by adenosine. Since the deamination rate constant of adenosine to inosine associated with ADA_1*2/*1 phenotype is slower than that associated with ADA_1*1/*1, the interaction of ADA_1 on ACP_1 activity may be explained by a lower intracellular concentration of inosine in ADA_1*2 carriers.

Material and Methods

A total of 1913 of subjects from the population of Central Continental Italy and a total of 1384 subjects from the population of Central Sardinia were examined.

We studied the following samples:

Children with Type 1 diabetes	286 subjects (Ref 6)
Women with Type 1 diabetes	107 subjects (Ref 7)
Women with repeated spontaneous abortion	170 subjects (Ref 4)
Allergic women with endometriosis	38 subjects (Ref 5)
Crohn's disease	70 subjects (unpublished)
Type 2 diabetes	273 subjects (Ref 3)
Subjects with Cardiovascular Diseases	339 subjects (unpublished)
Blood donors (2 samples)	290 subjects (unpublished)
Healthy newborn infants	340 subjects (Ref 1)
Children from the Central Area of Sardinia	1384 subjects (Ref 7)

ACP1 and ADA1 phenotype were determined as previously described [1-7]. Test of independence was carried by Chi-square and differences between means evaluated by Student-t. Three way contingency table to test interaction among variables was carried out by log-linear model. All analyses were carried out by commercial software (SPSS).

All subjects gave written informed consent to participate to the study that was conform with The Code of Ethics of the World Medical Association.

Results

Table 2 shows the proportion of low activity ACP_1 genotypes (*A/*A and*A/*B) in ADA_1*1/*1 subjects and in carriers of ADA_1*2 allele. Five classes of subjects with immunological disease and two samples of blood donors are reported. In all samples of immunological diseases the proportion of low activity ACP_1 genotypes is higher in carriers of ADA_1*2 allele than in ADA_1*1/*1 genotype. Overall the ODDS ratio is 1.967 (95%C.I. 1.278-3.030). The pattern of association is different from that observed in blood donors in whom the proportion of low activity ACP1 genotypes is higher in ADA_1*1/*1 genotype than in carriers of ADA_1*2 allele. There is a statistically significant interaction suggesting that ADA_1 genotype modifies the pattern of association between ACP_1 and the diseases considered: low activity ADA_1 could lower *A/*A and *A/*B activities and in turn ZAP 70 activity resulting in a weakening of T cell receptor signaling. An additive effect seems unlikely since a high concentration of adenosine, due to low activity of ADA_1 genotypes carrying the ADA_1*2 allele, would contribute to strengthen T cell activation (see table 1) with an effect opposite to that observed in association with low ACP_1 activity.

Table 3 shows the proportion of *A/*A and *A/*B genotypes carrying the ADA_1*2 allele in subjects with immunological diseases and in blood donors. These joint genotypes are much more frequent in the immunological diseases than in blood donors (p=0.0013; O.R. 2.31 95% C.I. 1.465-5.910) suggesting that these genotypes predispose to the diseases considered.

In fig 1 we compared patients with immunological diseases to blood donors and consecutive health newborns from the same population. The proportion of the joint genotypes *A/*A and *A/*B carrying the ADA_1*2 allele in healthy newborns shows an intermediate value between blood donors and immunological diseases. The data are compatible with the hypothesis that subjects with the genotypes *A/*A and *A/*B carrying the ADA_1*2 allele are more susceptible to post-natal diseases, thereby resulting in the exclusion of them from the possibility to be accepted as blood donors. Based on the difference observed between newborns and blood donors, we have calculated that the proportion of subjects unable to be accepted as blood donors because ADA_1-ACP_1 interaction is around 3%.

Table 4 shows the proportion of ACP_1*A/ADA_1*2 gametic type in relation to past malarial morbidity in the central area of Sardinia. The proportion of this gametic type is decreasing with increasing malarial morbidity thereby suggesting an increased susceptibility to severe manifestations of malaria in carriers of this gametic type resulting in a negative selection in areas of high endemia.

The observations reported in tables 2-4 suggest that the joint genotypes *A/*A and *B/*A carrying the ADA_1*2 allele are more susceptible to diseases involving the immune system as compared to other genotypes. The following data point to a positive role of this joint genotype on intrauterine growth and against some classes of diseases of post-reproductive period of life.

Fig. 2 shows the proportion of newborns with birth weight $\geq 90°$ centile, in relation to ACP_1 and ADA_1 genotypes in a sample of consecutive newborns (1). The proportion of these infants is higher in *A/*A and *A/*B genotypes carrying the ADA_1*2 allele than in other joint genotypes suggesting a favorable effect of these joint genotypes on intrauterine growth.

Table 5 shows the effect of the joint ACP_1-ADA_1 genotype on glycemic level in subjects with type 2 diabetes. The proportion of *A/*A and *A/*B subjects carrying the ADA_1*2 allele is lower in subjects with very high glycemic level than in subjects with a moderate increase of glycemic level. This finding suggests a protective effect of the joint *A/*A and *A/*B genotypes carrying the ADA_1*2 allele against extreme blood glucose levels. Since low ACP_1 activity (*A/*A and*A/*B activity) increases glucose tolerance and this is very marked in subjects carrying the ADA_1*2 allele the association could be related to the lowering of ACP_1 activity in the presence of ADA_1*2 allele, thus explain the highly significant interaction between ADA_1, ACP_1 and glycemia. A cooperative effect appears unlikely considering that a high adenosine concentration due to low ADA activity in ADA_1*2 carriers would bring about a high activity of adenosine receptors thereby resulting in a decreased glucose tolerance (see table 1). The increased glucose tolerance in low ACP_1 activity genotypes could be due to increased glucose metabolism connected with lower dephosphorylation of insulin receptor and B3P but also to decreased dephosphorylation of FMN that may increase the metabolic output due to increased activity of flavoenzymes in Krebs cycle.

Table 6 shows the relationship between the joint ACP_1/ ADA_1 genotypes and Left Ventricular Ejection Fraction (LVEF) in subjects with cardiovascular diseases. Both ACP_1 and ADA_1 activity have been found associated with cardiovascular diseases [27,28]: low ACP_1 activity and low ADA_1 genotypes have been found to exert a protective effect. The table shows that LVEF is significantly higher in the joint genotypes ACP_1 *A/*A and *A/*B carrying the ADA_1*2 allele than in other joint genotypes. This finding may represent an

example of cooperative interaction between the two systems.

Discussion

The finding described by Lucarini et al (2) indicates that in carriers of low activity ACP_1 *A/*A and *A/*B genotypes the presence of ADA_1*2 allele leads to a further decrease of enzymatic activity and this could be responsible for the associations observed at clinical level. However considering the effects of ACP_1 and ADA_1 on the immune response and on glucose metabolism a cooperative action is also possible (Table 1,[3,4]) . A cooperative action on immune reaction is supported by the well known relationship between ADA_1 and immune diseases and by the relationship between ACP_1 and T-cell activation. A cooperative action on metabolic functions is also possible and is suggested by the role of ACP_1 and adenosine receptors activity on glucose metabolism. As a protein phosphotyrosine phosphatase, ACP_1 may have an important role in modulation of glycolitic rate trough the control of insulin receptor activity and of band 3 protein phosphorylation status. Catalysing the conversion of FMN in riboflavin, the enzyme may have an important role in regulating flavo-enzyme activity and energy metabolism. Concerning ADA_1, recent studies have shown that adenosine counteracts insulin action in the liver by activating A2B receptors. Concerning ACP_1 possible effects on energy metabolism is also suggested by the action on ACP_1 on FMN.

The observations in tables 2 and 5 are compatible with the hypothesis of a further decreased activity of ACP_1 *A/*A and *B/*A genotypes in carriers of ADA_1*2 allele resulting in negative effects on ZAP70 and in turn on lymphocyte activity thus explaining the increased susceptibility to immunological diseases and malaria. Comparing the proportion of the joint genotype *A/*A and *B/*A carrying the ADA_1*2 allele observed in blood donors with that observed in newborns (fig 1) we have calculated that the effects of these joint genotypes on the health of individuals results in the exclusion of about 3% of the population from the category of blood donors.

A positive role of these joint genotypes has been observed in intrauterine growth, type 2 diabetes and subjects with cardiovascular diseases. The effects on intrauterine growth and on glycemic levels in T2D may be explained by the decrease of on ACP_1 *A/*A and *B/*A activity in carriers of ADA_1*2 allele resulting in an increase of insulin action, glucose utilization and metabolic activity. Since it has been observed that both low activity ACP_1 genotypes and low ADA_1 activity exert a protective effect against coronary artery diseases [26,27], a cooperative effect of these genotypes on cardiac function appears likely.

Some samples are small and the results need to be confirmed before drawing definitive conclusions.
A statistically significant association between ACP_1 and ADA_1 has been found in Caucasians living in Australia [28] and in a Brazilian population [29]. On the other hand, no significant association has been observed in other populations [30], suggesting some effects of genetic background and/or environmental conditions.

The frequency of ACP_1*A allele in human populations increases with decreasing distance from the North Pole [31]. Observations by our group suggest that the relative intrauterine survival rate of *A/*A genotype carrying the ADA_1*2 allele is greater at high than at low altitude [32]. Since the activity of ACP_1*A/*A is lower in the presence of ADA_1*2 than in its absence, the pattern observed represents a further argument in favor of the hypothesis that low ACP_1 activity has a selective advantage at low environmental temperatures.

Interactions between ACP_1 and ADA_1 with diverse intensity and direction of selective effects during intrauterine and reproductive stages of life would result in a statistical association of diverse strength and direction between the two genetic systems in later stages of life among human populations. Such effects persisting for generations may result in statistical correlations between gene frequencies at corresponding loci. In this context, the observation of Farabegoli and Barbujani [33] that the axis of the gradient for ACP_1 alleles, parallels the gradient observed for ADA locus, is of interest.

The joint system ACP_1-ADA_1 seems to have an important role in human health and development. The ACP1 (*A/*A and *B/*A) genotypes carrying the ADA_1*2 allele may be advantaged during intrauterine life through a positive effect on fetal growth. During pre-reproductive and early reproductive stages of extra uterine life, individuals carrying these joint genotypes may be disadvantaged since they could be more susceptible to diseases involving the immune system. In the post-reproductive period of life these genotypes may be relatively protected against common diseases occurring during the last period of human life.

The observation on Type 2 diabetes is in agreement with experimental data showing that the reduction of ACP_1 activity increases glucose tolerance [34], thereby pointing to possible beneficial effects of a pharmacologic reduction of ACP_1 activity in diabetic patients.

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Table1. Mechanism by which ADA_1 and ACP_1 activity may influence immune response and glucose metabolism

		Effect on immune response	Effect on glucose metabolism
Low concentration of adenosine due to high ADA activity(ADA_1*1/*1)	Low activity of adenosine receptors	Decreased T cell activity	Increased glucose tolerance
High concentration of adenosine due to low adenosine deaminase activity (carriers of ADA_1*2 allele)	High activity of adenosine receptors	Increased T cell activity	Decreased glucose tolerance
Low dephosphorylating activity on the negative regulatory Tyr 292 of ZAP70 and on tyrosine kinase receptors (ACP_1*A/*A and *A/*B)	Low ZAP-70 activity and high activity of tyrosine kinase receptors	Decreased T cell activity	Increased glucose tolerance
High dephosphorylating activity on the negative regulatory Tyr292 of ZAP70 and on tyrosine kinase receptors (other ACP_1 genotypes)	High ZAP-70 activity and low activity of tyrosine kinase receptors	Increased T cell activity	Decreased glucose tolerance

Table 2. Proportion of low activity ACP1 genotypes *A/*A and *A/*B in ADA1*1/*1 subjects and in carriers of ADA*2 allele. Comparison between blood donors and subjects with immunological diseases.

		%proportion of *A/*A and *A/*B	**Total n°**	**O.R.**
IMMUNOLOGICAL DISEASES				
Types 1 diabetes Children	ADA_1*1/*1 Carriers of ADA_1*2	47.4% 66.6%	232 54	2.21
Types 1 diabetes Adults	ADA_1*1/*1 Carriers of ADA_1*2	37.1% 50.0%	89 18	1.70
Women with repeated spontaneous abortion	ADA_1*1/*1 Carriers of ADA_1*2	49.0% 70.6%	153 17	2.50
Allergic women with endometriosis	ADA_1*1/*1 Carriers of ADA_1*2	36.7% 62.5%	30 8	2.90
Crohn's disease	ADA_1*1/*1 Carriers of ADA_1*2	28.3% 41.2%	53 17	1.77
All diseases	ADA_1*1/*1 Carriers of ADA_1*2	43.5% 60.5%	557 114-	1.97 95%C.I. 1.28-3.03

BLOOD DONORS				
Sample 1°	ADA_1*1/*1 Carriers of ADA1*2	49.1% 20.0%	171 30	0.26
Sample 2°	ADA_1*1/*1 Carriers of ADA1*2	43.3% 22.7%	67 22	0.38
All blood donors	ADA_1*1/*1 Carriers of ADA_1*2	47.5% 21.1%	238 52	0.30 95%C.I. 0.14 – 0.63

Statistical analysis : Three way contingency table analysis by a log linear model
X=ACP_1 y=ADA_1 z=Sample

	G	df	p
IMMUNOLOGICAL DISEASES			
xyz interaction	0.526	4	0.970
xy independence	13.249	5	0.025
xz independence	15.635	8	0.070
yz independence	12.850	8	0.120
BLOOD DONORS			
xyz interaction	0.285	1	0.650
xy independence	12.546	2	0.002
xz independence	0.716	2	0.700
yz independence	3.471	2	0.180
ALL IMMUNOLOGICAL DISEASES VS ALL BLOOD DONORS			
xyz interaction	22.948	1	<0.0002
xy independence	23.506	2	<0.0001
xz independence	24.206	2	<0.0001
yz independence	23.094	2	<0.0001

Table 3. Proportion of ACP_1 *A/*A and *A/*B genotypes carrying the ADA_1*2 allele. Comparison between blood donors and subjects with immunological diseases

	%Proportion of *A/*A and *A/*B subjects carrying ADA_1*2 allele	Total n°
IMMUNOLOGICAL DISEASES		
Types 1 diabetes Children	12.6%	286
Types 1 diabetes Adults	8.4%	107
Women with repeated spontaneous abortion	7.1	170
Women with allergic endometriosis	13.1%	38
Crohn's disease	10.0%	70
All diseases	10.3%	671
Chi square test of independence		
χ^2 *4.314*	*df* *4*	*p* *0.365*
BLOOD DONORS		
Sample 1°	3.0%	201
Sample 2°	5.6%	89
All blood donors	3.8%	290
Chi square test of independence		
χ^2 *0.561*	*df* *1*	*p* *0.454*
Comparison all immunological diseases vs all blood donors		
10.341	*1*	*0.0013*
O.R. 2.91	*95% C.I. 1.46-5.91*	

Table 4 . Proportion of ACP_1 *A /ADA_1*2 gametic type in relation to past malarial morbidity in the central area of Sardinia (from Ref. 7 modified).

	malarial morbidity %	Altitude –meters above sea level	Proportion of *A/ADA_1*2 gametic type	Total n° of gametic types
Low altitude	82%	10-45	0.4%	786
Medium altitude	29%	185-648	1.1%	1262
High altitude	17%	796-1000	1.8%	720
Chi square test of independence	χ^2 *7.188*	*df* *2*	*p* *0.027*	

Table 5. The effect of ACP_1 and ADA_1 on glycemic level in T2D.

Glycemia	ACP_1 genotype	Proportion of ADA_1*2 carriers	Total n°
≤160	*A/*A and *A/*B Other genotypes	19.8% 15.8%	96 120
>160	*A/*A and *A/*B Other genotypes	4.8% 31.6%	21 36
Three way contingency table analysis x=ADA_1; y=ACP_1; z=Glycemia xyz interaction p=0.001			

Table 6. Left Ventricular Ejection Fraction in subjects with cardiovascular diseases

ACP1 genotype	Ejection Fraction		
	Mean	S.E.	Total n°
*A/*A and *A/*B genotypes carrying ADA_1*2 allele	57.29	1.34	17
Other joint genotypes	51.93	0.52	322
Student-t for difference between means t 3.747 p 0.001			

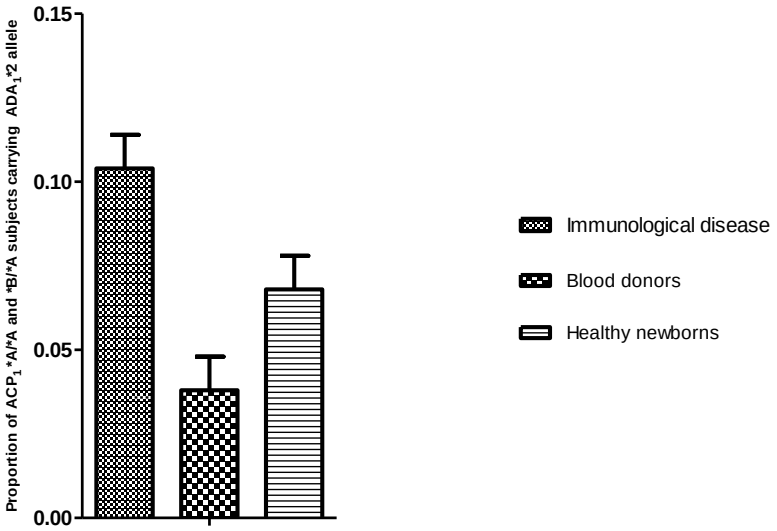

Fig.1. Proportion of ACP1 *A/*A and *B/*A genotypes carrying the ADA1*2 allele. Comparison among patients with immunological diseases, helathy adults and healthy newborns from the same polulation: p=0.002.

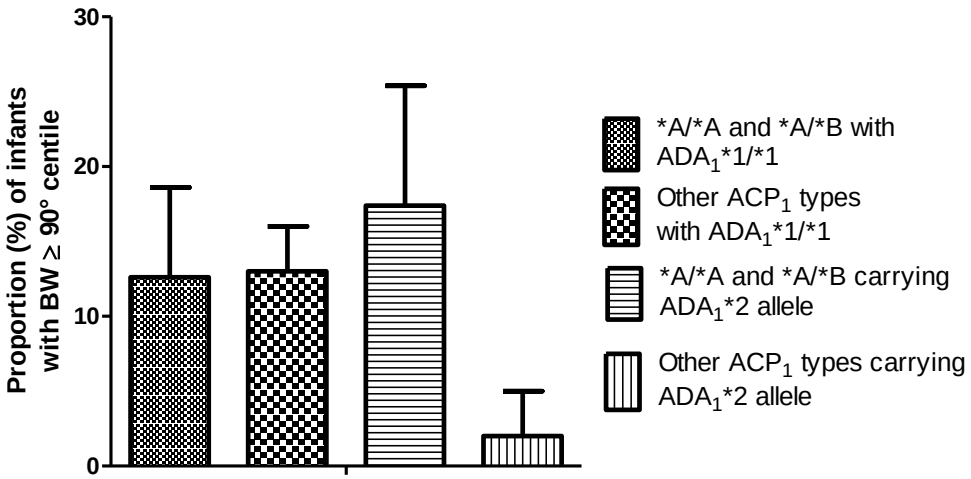

Fig 2. Proportion (%) of newborns with BW ≥ 90° centile in relation to ACP1 and ADA1genotypes. Interaction between ACP1, ADA1 and BW ($p<0.02$).