9-1-2012

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The Effect of ACP_1-ADA_1 Genetic Interaction on Human Life Span

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Running title: ACP_1-ADA_1 interaction and life-span

KEY WORDS: ACP_1, ADA_1, LONGEVITY, GENETIC INTERACTION, SIRTUIN GENES

Abstract

Acid phosphatase (ACP_1) is a polymorphic enzyme which catalyzes the conversion of flavin-mononucleotide (FMN) to riboflavin and regulates the cellular concentration of flavin-adenine-dinucleotide (FAD) and, consequently, energy metabolism. Its activity is modulated by adenosine deaminase (*ADA_1*) genotype. Aim of our work is to verify whether individuals with a high proportion of ACP_1 *f* isozyme and carrying *ADA**2 allele, displaying the highest phosphatase activity, may have a higher life expectancy.

Genomic DNA was extracted from peripheral blood of 569 females and 509 males (18-106 years) randomly recruited from Central Italy. These samples were subdivided into three sex-specific age groups (the ages of women are in square bracket): Class 1:age <66 [<73]; Class 2: age 66-88 [73-91]; Class 3: age >88 [>91]. *ACP_1*and *ADA_1* SNPs were genotyped by RFLP-PCR methods and statistical analyses were performed using SPSS 14.0. The results showed a larger proportion of Class 3 individuals displaying high ACP_1 *f* isozyme concentration and carrying *ADA_1**2 allele than those of Class 2 and Class 2+1. Thus, we postulate that in Class 3 individuals the high phosphatase activity, resulting from the combined presence of high ACP_1 *f* isozyme concentration and the *ADA_1**2 allele, lowers the rate of glycolysis which may reduce the amount of metabolic calories and, in turn, activate Sirtuin genes that protect cells against age-related diseases.

Introduction

ACP1 gene (gene map locus 2p25) encodes for the ACP_1 polymorphic enzyme also called Low Molecular Weight Protein Tyrosine Phosphatase (LMW-PTP). Two functions have been suggested for ACP_1: flavin-mononucleotide (FMN) phosphatase and tyrosine phosphatase. As flavin–mononucleotide phosphatase, ACP1 catalyzes the conversion of FMN to riboflavin, thus regulating cellular flavin-adenine-dinucleotide (FAD) concentration, flavo-enzyme activity and energy metabolism. As phosphotyrosine phosphatase, ACP_1 may modulate the glycolytic rate controlling insulin receptor activities and band 3 protein status.

ACP1 shows three common codominant alleles, *ACP1**A, *ACP1**B and *ACP1**C, whose combinations define six phenotypes characterized by different enzyme activity (in the order, A<B/A<(B, C/A)<C/B). The ACP_1 C phenotype is very rare being present only in Caucasian people and it displays the highest enzyme activity (Boivin and Galand 1986; Bottini et al. 2002; Fuchs et al. 1992; Spencer et al. 1964).

Low ACP_1 activity makes an individual more susceptible to allergic disorders, to Th1-immune diseases, to type 1 diabetes and Crohn's disease, and lowers the age at onset (≤ 6yr) of type 1 diabetes. *ACP1* *A/*A and *B/*A genotypes are over-represented in children with idiopathic generalized tonic-clonic seizures, suggesting a detrimental role of low ACP_1 activity also in central nervous system. *ACP1**A allele has the highest frequencies in the population of northern latitudes and it has been associated with greater body size and with adaptation to cold stress. ACP_1 A and ACP_1 B/A phenotypes are also associated with severe body mass increase in obese adult subjects, in obese diabetic pregnant women and in normal children (Bottini et al. 1990; Bottini et al. 2002a; Bottini et al. 2002b; Bottini et al. 2007; Gloria-Bottini et al. 2007; Greene et al. 2000; Lucarini et al. 1990; Paggi et al. 1991).

The *ACP*$_1$ gene expresses two isozymes (*f* and *s)* showing different concentrations among the six ACP$_1$ phenotypes. These isozymes are expressed simultaneously in many tissues and they are characterized by different biological functions. ACP$_1$ *f* isozyme is the main responsible of the total ACP$_1$ activity (Dissing and Svensmark 1990; Fujimoto et al. 1988; Stefani et al. 1993). Since different effects on *f* and *s* isozymes activity result from modulation of ACP$_1$ enzymatic activity, C, CA and A phenotypes - characterized by lower concentrations of *f* isozymes - could be more susceptible to damage by oxidative events compared to the other phenotypes. Notably, leiomyoma size is negatively correlated with ACP$_1$ *f* isozyme concentrations regulating negatively cell proliferation and growth of leiomyomas through dephosphorylation of the PDGF receptor (Ammendola 2009).

Adenosine deaminase locus 1 (ADA$_1$) is a polymorphic enzyme catalyzing the irreversible deamination of adenosine to inosine. It is present in all mammalian tissues and it is controlled by a locus on the long arm of chromosome 20 with two co-dominant alleles *ADA$_1$*1 and *ADA$_1$*2 having different associated enzymatic activities. *ADA$_1$*1 allele displays 30% higher enzymatic activity than *ADA$_1$*2 allele (Battistuzzi et al. 1974). Experimental studies have also shown that adenosine, acting via adenosine 1 receptor, increases insulin sensitivity in isolated adipocytes and decreases insulin sensitivity in isolated muscle fibers (Challis et al. 1992; Dunwiddie and Masino 2001; Vannucci et al. 1992). Interest has been focused on a wide variety of effects produced by adenosine through the activation of cell surface adenosine receptors (Richardson 1997; Xu et al. 1998; Yasuda et al. 2003). Transient activation of adenosine receptors protects against damage following hypoxic or ischemic events in brain and in other excitable tissues such as heart. Variation in energy states, cardiac stress or other stimuli induce the release of adenosine which by its receptors can lead to a more efficient balance between energy utilization and energy supply, also protecting cardiac cells under extreme stress conditions (Headrick et al. 2011).

ACP_1 enzyme activity is modulated by *ADA_1* genotype: in carriers of *ADA_1**2 allele, ACP_1 A, BA, CA and CB activity is lower than in homozygous for *ADA_1**1 allele (Lucarini et al. 1989). A positive association of the genotype *ACP_1**A/*A and *ADA_1**2 allele with type 1 diabetes and a negative correlation between the frequency of this gametic type with past malarial morbidity in Sardinia (Gloria-Bottini et al. 2010) have been already reported. Moreover, a significant association between ACP_1 and ADA_1 has been found in Caucasians living in Australia and in a Brazil (Engràcia et al. 1991)

Since ACP_1 *f* isozyme is the main responsible of ACP_1 activity modulated by *ADA_1* we tested whether the subjects with a high proportion of ACP_1 *f* isozyme and carrying *ADA_1**2 allele have an higher life-expectancy.

Materials and Methods

Subjects

Peripheral blood was obtained from 1072 (569 females and 503 males) unrelated individuals, 18–106 years old, randomly recruited from the same geographical area of Central Italy (Marche region) on the eastern side of the Apennines. The whole population studied was composed by Caucasian individuals all descendants of an ancient pre-Roman Italian population called the *Piceni* (Cavalli-Sforza et al. 1994). The same donors provided information concerning their health condition. No pathological condition existed (e.g. cancer, diabetes, heart diseases, hypertension, obesity, and chronic inflammatory diseases). The sample study was divided into three sex-specific age classes (the age classes of women are written in square bracket) The first age class was made by men with age <66 [<73], the second class by men with age 66-88 [73-91], and the third class by men with an age >88 [>91]. These gender-specific age classes were defined according to demographic information and accounted for different survivals of men and women in Italian population (Passarino et al. 2006). The study protocol was approved by the Joint Ethical Committee (JEC) University of Camerino-Azienda ASUR Marche ZT-10 Camerino, in accordance with the Declaration of Helsinki in its revised edition and with international and local regulatory requirements.

Genotyping

Genomic DNA extraction was carried out from peripheral blood through standardized salting out method and DNA was stored at −20 °C until gene analysis.

ACP_1 and *ADA_1* SNPs were genotyped according to RFLP-PCR methods previously published (Lazaruk 1995; Napolioni and Lucarini 2010). For *ACP_1,* all PCRs were set up in 30 µl and 0.2 µmol/L of both primers, 0.1 mmol/L dNTPs, 1.5 mmol/L MgCl2, 0.5 U of Taq

polymerase (AmpliTaq, Applied Biosystem, Mannheim, Germany), 1X AmpliTaq buffer (PE), and 50 ng of DNA template. The amplification conditions consisted of an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 45 seconds, 54°C for 45 seconds, 72°C for 45 seconds, and a final extension at 72°C for 5 minutes. The annealing temperature, extension time, and primer concentration for the 2-kb amplification product were 57°C, 120 seconds, and 0.1 μmol/L, respectively. Oligonucleotides used for PCR amplification are reported in Table 1. The C>T transition at codon 43 and the A>G transition at codon 105 generate a *CfoI* and a *TaqI* restriction site that, together, were used for PCR-based genotyping, respectively. A 341-bp segment spanning the entire exons 3 and 4 was amplified using primers 263 to 264 (Table 1). A 299-bp segment including exon 6 was amplified using primers 267 and 268. Ten microliters of the 341-bp exon 3 amplicon was fully cleaved by *CfoI* at 37°C for 1 hour according to the manufacturer's instructions and then electrophoresed on 1.8% agarose gels. The digestion created two fragments of 255 and 86 bp for the ACP_1*A and ACP_1*B haplotype, whereas the ACP_1*C haplotype was not cut. Similarly, the 299-bp PCR product was digested by *TaqI* at 65°C for 1 hour according to the manufacturer's instructions, generating 2 fragments of 100 and 199 bp for the ACP_1*A haplotype but not for the *B and *C haplotypes.

Briefly, for ADA_1, PCR was performed with primers flanking the 22G>A polymorphic region: 5'-GCCCGGCCCGTTAAGAAGAGC-3' as sense primer, and 5'-GGTCAAGTCAGGGGCAGAAGCAGA-3' as antisense primer. The PCR products were digested with *TaqI* endonuclease for 90 min at 65°C, and the samples were electrophoresed in a 1.8% agarose gel. ADA_1 22*A (ADA_1*2) allele was identified by the lack of a *TaqI* restriction site.

Statistical analysis

Haplotype and genotype frequency were calculated by genotype counting method. Hardy–Weinberg Equilibrium (HWE) was assessed by comparing the genotype frequencies with

the expected values using a contingency table χ^2 statistics. Means and T-test for difference between means were carried out by using the SPSS programs.

Results

Table 2 shows the frequency of *ACP1* genotypes in relation to *ADA1* genotypes and classes of age in males and females separately. All age classes are in Hardy-Weinberg Equilibrium. In both sexes *ACP1* *A/*B and *B/*B – *ADA1* genotypes have a greater frequency in all age classes.

Table 3 shows *f* and *s* *ACP1* genotype isozyme concentrations according to Dissing (Dissing 1987, 1993). The data of Dissing are reported to clarify the procedure to obtain the data reported in Table 4.

Table 4 shows the concentration of ACP_1 *f* isozyme - *ADA1**2 allele along the three age classes studied. The mean value of ACP_1 *f* isozyme amount among the various ACP_1-ADA_1*2 genotypes is reported for each class (Dissing 1987, 1993). It is also reported the T test for differences between means. The concentration of ACP_1 *f* isozyme - *ADA1**2 allele in Class 3 subjects is higher than the one in Class 2 and Class 1, but, compared to the Class 1, it does not reach statistical significance (p=0.053). ACP_1 *f* isozyme - *ADA1**2 allele concentration of subjects in Class 1 is not different from that of subjects in Class 2 while the differences between the concentration in Class 3 and Class 2 and between Class 3 and Class 2 + Class 1 are highly significant.

Discussion

In Class 3 compared to Class 2, or to Class 2 plus Class 1, there is a significant excess of individuals displaying higher amount of ACP_1 *f* isozyme – ADA_1 *2 allele (table 4). Boivin and Galand (1986) demonstrated that ACP_1 dephosphorylates the tyrosine residues of the erythrocyte membrane protein 3 (B3P). The phosphorylation of B3P tyrosines prevents the binding of several glycolytic enzymes, causing high glycolytic rates in erytrocytes (Harrison et al. 1991; Low et al. 1987). Furthermore, Stefani et al. (1993) demonstrated that the phosphotyrosine of a syntethic peptide corresponding to the sequence 5-16 of the B3P is much more efficiently hydrolyzed by the ACP_1 *f* isozyme than *s* isozyme. Therefore, as the ACP_1 *f* amount is strongly related to ACP_1 activity (Dissing and Svensmark 1990; Stefani et al. 1993) and its activity is enhanced by ADA_1*2 (Lucarini et al. 1989), it is conceivable that this fact may result in a significantly dephosphorylation of tyrosine residue of B3P, slowing glycolytic rates in erythrocytes. Therefore, this may affect the Krebs cycle resulting in a lesser amount of metabolic calorie. Moreover, the highest ACP_1 activity can dephosphorylate insulin receptor, counteracting the action of insulin and decreasing glucose utilization.

Calorie restriction in mammals activate the Sirtuin genes (Baur et al. 2010; Bordone and Guarente 2005; Civitarese et al. 2007; Cohen et al. 2004; Imai and Guarente 2010; Kelly 2010; Qiu et al. 2010) which protect cells against age-related diseases such as, cancer, atherosclerosis, cardiovascular diseases, neurological disorders and diabetes (Haigis and Guarente 2006; Haigis and Sinclair 2010; Kim and Um 2008; Kim et al. 2007; Lagouge et al. 2006; Milne et al. 2007; Westphal et al. 2007). We suggest that subjects displaying higher amount of ACP_1 *f* isozyme and carrying ADA_1*2 allele probably display a higher life-expectancy for a lower amount of metabolic calories. Moreover, higher concentration of both circulating and intra-cellular adenosine (Battistuzzi et al. 1974) can enjoy positive effects on neural and cardiac tissues during hypoxic or ischaemic

events (Cohen and Downey 2008; Headrick et al. 2011; Latini and Pedata 2001; Mubagwa and Flameng 2001; Peaeson et al 2003; Pedata et al. 2007).

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Table 1. Primers used for ACP_1 SNPs analysis

Primer	*Target Amplification*	*Nucleotide Sequence 5'-3'*
#263	Exon 3	AGGCCACCTGAACTCCTCT
#264	Exon 3	CCTGTCTTGTTTATGGGCT
#267	Exon 6	TTCAGAAGACCCTAGCAGATG
#268	Exon 6	TGGCAAAACCTGCATAACAA

Abbrevation: SNPs, single-nucleotide polymorphisms.

Table 2. Distribution of *ACP_1* genotype in relation to *ADA_1* and classes of age in females and males

		*ADA_1 *1/*1*			*ADA_1 *2 carriers*		
Sex	*ACP_1*	*Age Classes*			*Age Classes*		
		1	2	3	1	2	3
Females	*A/*A	4.8%	14.7%	7.2%	7.0%	11.4%	---
	*B/*A	47.3%	41.5%	42.2%	34.9%	34.1%	41.2%
	*B/*B	41.1%	41.9%	41.0%	44.2%	43.2%	52.9%
	*C/*A	2.1%	2.5%	2.4%	7.0%	4.5%	---
	*C/*B	4.8%	9.3%	7.2%	7.0%	6.8%	5.9%
	*C/*C	---	---	---	---	---	---
Total n°		146	236	83	43	44	17
ACP_1 Hardy-Weinberg Equilibrium (p)		Class 1 0.226;	Class 2 0.325;	Class 3 0.730			
ADA_1 Hardy-Weinberg Equilibrium (p)		Class 1 0.270;	Class 2 0.513;	Class 3 0.845			
Males	*A/*A	6.3%	5.8%	6.5%	---	11.8%	---
	*B/*A	38.3%	35.6%	37.0%	50.0%	41.2%	25.0%
	*B/*B	43.0%	43.3%	42.4%	37.5%	41.2%	75.0%
	*C/*A	6.3%	6.7%	3.3%	6.3%	3.9%	---
	*C/*B	4.7%	8.7%	10.9%	6.3%	2.0%	---
	*C/*C	1.6%	---	---	---	---	---
Total n°		128	208	92	16	51	8
ACP_1 Hardy-Weinberg Equilibrium (p)		Class 1 0.057;	Class 2 0.115;	Class 3 0.936			
ADA_1 Hardy-Weinberg Equilibrium (p)		Class 1 0.896;	Class 2 0.268;	Class 3 0.953			

Table 3. *f* and *s* isozyme concentrations in relation to ACP_1 genotype (as reported in Dissing 1987, 1993). The quantities of enzyme are given per millilitre of packed red cells, RBC indicates red blood cells.

Total quantity of f (μg/mL RBC)		*Total quantity of s (μg/mL RBC)*	
*B/*B	16.4	*C/*C	20.6
*B/*A	12.0	*C/*A	12.7
*C/*B	11.3	*C/*B	12.1
*A/*A	7.9	*B/*B	3.9
*C/*A	7.5	*B/*A	3.4
*C/*C	5.7	*A/*A	3.3

Table 4. Concentration of *ACP_1 f* isozyme-*ADA_1**2 allele in relation to age classes

	ACP_1 f isozyme-*ADA_1**2 allele			
Age classes	*Mean*	*S.D.*	*S.E.*	*Total n°*
Class 1	13.3	2.99	0.39	59
Class 2	13.16	3.12	0.32	95
Class 3	14.61	2.24	0.45	25

T-test for differences between means (p)	
Class 2 vs. Class 1	0.777
Class 3 vs. Class 1	0.053
Class 3 vs. Class 2	0.031
Class 3 vs. Class 2+1	0.030