The genetics of signal transduction and the outcome of diagnostic tests in growth retardation

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Abstract

The effects of 'normal' genetic variability of signal transduction on endocrine function may be more evident during stimulation tests than is observed in basal states, thereby contributing to a greater understanding of the possible role of signal transduction genetics in the pathogenesis of endocrine disorders. In the present study, we have studied the outcome of growth hormone (GH) stimulation testing by insulin in growth-retarded children in relation to the genotype of ACP1 (acid phosphatase locus 1; also referred to as cLMWPTP, cytosolic low molecular weight phosphotyrosine phosphatase). ACP1 is an enzyme, expressed as two distinct isoforms designated F and S, that down-regulates insulin receptor signal transduction and which shows a genetic polymorphism with

strong quantitative enzymatic differences among genotypes. In this study, we examined 116 growth-retarded children of which 101 were genotyped for ACP1. We found that the basal level of GH is higher in ACP1 genotypes with low concentrations of the S isoform than in genotypes with high S isoform concentrations ($P < 0.02$). Additionally, during GH stimulation with insulin, the genotypes with low S isoform concentrations were found to perform better ($P < 0.005$) and to react more promptly than the genotypes with high S isoform concentrations ($P < 0.05$). These findings suggest that high S isoform ACP1 activity slows down the effect of insulin, resulting in a retardation of its metabolic effect.

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Introduction

Based on the outcome of growth hormone (GH) stimulation tests, growth retardation in children is commonly classified into three categories: familial short stature, partial deficit and total deficit of GH. It is likely that such classification, although useful in clinical practice, does not correctly reflect the multifactorial origin of the great majority of these disorders. A multivariate continuous distribution of relevant auxologic and endocrine parameters may be more realistic, while incorrect representation may, in part, account for the poor reproducibility of clinical categorical definitions (Rose *et al.* 1988, Cacciari *et al.* 1992).

It is likely that many factors are involved in the determination of relevant clinical parameters of growth retardation. Some of these factors may be intrinsic to GH genetic differences, while others may be involved in receptor and signal transduction mechanisms. However, the effects of 'normal' genetic variability of signal transduction on basal endocrine function may not discern

important differences that are detectable following stimulation tests, a fact which led to the present study.

Acid phosphatase locus 1 (ACP1) is an enzyme involved in the signal transduction of insulin and other growth factors (Dissing 1993, Bottini *et al.* 1995, Chiarugi *et al.* 1997, Ramponi & Stefani 1997). ACP1 is encoded at a locus on chromosome 2 showing three common alleles *A, *B and *C (Dissing 1993, Bottini *et al.* 1995). The enzyme is expressed in mammals as two distinct isoforms, designated F and S with reference to their electrophoretic mobility. There are quantitative differences in total enzymatic activity and F/S ratio among ACP1 genotypes (see Bottini *et al.* 1995 for a review). *In vitro* experiments have shown that ACP1 down-regulates insulin receptor signal transduction (Chiarugi *et al.* 1997), and the ACP1 polymorphism has been found to be associated with glycemic levels in all forms of diabetes (Gloria-Bottini *et al.* 1996, Lucarini *et al.* 1998). These observations prompted us to examine the possible effect of ACP1 enzymatic variability on the results of GH stimulation by insulin in growth-retarded children.

Table 1 Clinically relevant variables in study sample

	Age (years)	Deviation from mean stature (in S.D.)	Maternal stature (cm)	Paternal stature (cm)	Sex ratio (M/F)
FSS					
Mean	11.3	− 2.11	152.9	164.3	2.00
S.D.	2.6	0.73	4.4	4.7	
n	30	27	28	27	30
PD					
Mean	10.3	− 2.08	154.3	168.6	2.41
S.D.	3.3	0.96	5.9	6.4	
n	57	48	55	54	58
TD					
Mean	9.9	− 2.00	154.5	168.5	1.55
S.D.	4.7	0.86	7.1	6.9	
n	28	23	27	26	28

Subjects and Methods

One hundred and sixteen growth-retarded children in the population of Ancona, Italy have been studied; the ACP1 genotype was determined in 101 of these children. Insulin and clonidine stimulation tests were performed in all subjects. The GH value (plasma level in µg/l) at the end of the test was considered for classification into three categories: total deficit (TD), partial deficit (PD) and familial short stature (FSS). For insulin stimulation the cut-off points were 4 (PD vs TD) and 8 (FSS vs PD) µg/l for the level of GH at 60 min from the beginning of the test and for clonidine testing 6 and 10 µg/l respectively at 30 min from the beginning of the test were used. Both test results had to be below the cut-off point in order to include the patient in the lower class. Based on the results of GH stimulation tests with insulin and clonidine, among children in whom ACP1 genotype was determined 23 were classified as FSS, 54 as PD and 24 as TD of GH. With the exception of two subjects (one TD and one PD) who showed an associated deficit of thyrotropin (TSH), all cases had isolated GH deficiency. No other evident cause of growth retardation such as malabsorption was present, and no sign of sellar area lesion was detectable.

ACP1 genotyping was determined according to the classical method of Hopkinson *et al.* (1963). Indeed, only recently was a method developed which allows the determination of all ACP1 types by DNA analyses (Lazaruk 1995). In our laboratory, we have recently determined ACP1 types by the two methods (classical and by DNA analysis) in a sample of diabetic subjects and their parents. The results by the two methods were very similar. For our analyses in the present study, subjects were subdivided into two categories: low-medium ACP1 activity (*A/*A, *A/*B and *B/*B) and high activity (*A/*C, *B/*C and *C/*C). The first class includes genotypes with very low

S isoform concentrations (<4 µg/ml red blood cells) and the second class those with high S isoform concentrations (>12 µg/ml red blood cells) (Bottini *et al.* 1995).

Statistical analyses were performed by SPSS programs (SPSS/PC+ version 5.0 1995; SPSS Inc., Chicago, IL, USA). Probabilities in Table 2 were combined according to the method described by Sokal and Rohlf (1981). Informed consent was obtained by the parents of children prior to testing. The investigation was performed during the years 1985–1995 in collaboration between the Laboratory of Genetics of the University of Camerino and the Institute of Pediatric Clinic of the University of Ancona. It is noteworthy that, at present, insulin challenge testing is no longer used in Italian Pediatric Clinics.

Results

Clinically relevant parameters of the study sample are shown in Table 1. Table 2 shows the basal level of plasma GH in genotypes with high and low ACP1 S isoform concentrations. We found that the level of GH is higher in children with low S isoform concentrations than in those with high S isoform concentrations (*P*<0.02). No statistically significant difference in the deviation from normal stature has been observed between children with high and low S isoform concentrations.

Figure 1 shows the results of GH stimulation by insulin in ACP1 genotypes with low S isoform concentrations and in those with high S isoform concentrations separately for FSS and PD subjects. TD was not considered for this analysis since the increase of GH during the stimulation test was minimal. These data show that low S genotypes performed better than high S genotypes in both FSS and PD (cumulative *P*<0.005). The results obtained by stimulation by clonidine are similar to those obtained by insulin

Table 2 Basal level of plasma GH ($\mu\text{g/l}$) in genotypes with low and in those with high ACP1 S isoform concentrations

	*A/*A, *A/*B and *B/*B genotypes (low S)	*A/*C, *B/*C and *C/*C genotypes (high S)	Significance of difference (P)
FSS			
Mean	4.6	2.1	0.015
S.E.	0.9	0.0	
n	22	1	
PD			
Mean	3.4	3.1	0.700
S.E.	0.4	0.9	
n	46	8	
TD			
Mean	3.9	2.1	0.045
S.E.	0.8	0.1	
n	25	2	
Combined probabilities*			<0.02

*Probabilities were combined according to the method of Sokal & Rohlf (1981).

but the differences between low and high S genotypes are much less marked and statistically not significant (data not shown).

In both FSS and PD subjects the rise of plasma GH in the initial period of stimulation by insulin is slower in subjects with high S than in those with low S activity. In subjects with low S activity, the maximum plasma GH level is reached within 30–45 min, while in subjects with high S activity the maximum is reached after 45–60 min, at a time when the plasma GH level is already decreasing in subjects with low S activity. Table 3 shows a cumulative analysis of data in FSS and PD subjects. The difference in GH plasma level between low and high S activity genotypes is statistically significant only during the first 30 min.

A correlation analysis between the concentration of S (and F) isoform of ACP1 and the rise of plasma GH at 30 min from insulin infusion has shown that the S isoform

is negatively correlated with the rise of GH, while the F isoform does not exert any apparent influence on this rise (data not shown).

In the sample of PD cases we generated a polynomial approximation of the functional relationship between GH level and time in low S activity subjects. The function best fitting the rise of GH is a four order polynomial: $Y = 0.371x^4 - 3.04x^3 + 7.03x^2 - 2.56x$ (where Y is GH plasma level and x = time in min). After transformation of the data, the relationship between plasma GH level and time becomes linear in low S activity but not in high activity subjects (data not shown). This confirms the differences between low S and high S genotypes with respect to their impact on the relationship between time and rise of plasma GH during stimulation.

It is also noteworthy that the pattern of GH response to stimulation with insulin and clonidine was not found to have significant differences between sexes or between age classes.

Table 3 The rise of plasma GH after stimulation with insulin in subjects with low S concentration and in subjects with high S concentration. Cumulative analysis on FSS and PD subjects. The zero hypothesis is that there is no difference between low and high S concentration ACP1 genotypes

	Difference between low and high S concentration ACP1 genotypes	
	<30 min PII	>30 min PII
Mean value of difference	3.60	2.70
S.E.	1.32	1.58
No. of subjects	77	77
P-value	<0.05	N.S.

N.S., not significant. PII, post insulin infusion.

Discussion

Experimental evidence indicates that F and S isozymes elicit structural differences in their catalytic sites and have different cellular target substrates. It is likely, therefore, that they perform different physiological functions. Indeed, Stefani *et al.* (1993) have shown that S and F isoforms have somewhat different interactions with the insulin receptor. This may explain the differences observed in our study between high and low S ACP1 genotypes.

The role of many protein tyrosine phosphatases (PTPases) in modulating insulin receptor signal transduction has

Figure 1 Rise of plasma GH concentrations after stimulation with insulin in FSS subjects (upper panel) and in PD subjects (lower panel) with low activity (*A/*A, *A/*B and *B/*B; Δ) and with high activity (*A/*C, *B/*C and *C/*C; $\blacktriangle$) ACP1 genotypes. The number of subjects is shown in parentheses. The cumulative test of significance for the difference between genotypes with low and high S isoform concentrations is $P < 0.005$.

been demonstrated by *in vitro* experiments (Goldstein *et al.* 1998), and recently the *in vivo* role of PTPases in the modulation of insulin action has been investigated by the generation of a knock-out mouse model for one of these enzymes (Elchebly *et al.* 1999). Among the PTPases involved in insulin receptor signal transduction, ACP1 is the only one associated with 'normal' variability of enzymatic activity due to a known genetic polymorphism. The present data seems to confirm the *in vivo* involvement of ACP1 in insulin signal transduction, suggesting that ACP1 genetic variability influences the outcome of stimulation testing: high PTPase activity appears to slow down the effects of insulin, resulting in retardation of its metabolic effects. In turn, the fact that basal levels of plasma GH in all classes of patients were found to be higher in low S than in high S ACP1 genotypes also suggests that in physio-

logical conditions ACP1 activity may correlate negatively with GH plasma levels.

The differences observed between insulin and clonidine effects suggest that the pathway of clonidine action may not be as strictly dependent on the phosphorylation cascade as that of insulin action. That is, the effects of ACP1 activity are much stronger with insulin than with clonidine pathway activation.

In our subjects no direct measurements of glucose response level to infusion of insulin have been performed. Therefore, although the infusion may represent an insulin tolerance test, since the GH response is further removed from the insulin effects, at present it cannot be excluded that some effect of ACP1 is acting at an intermediate step of the pathway, including a possibly direct effect on pituitary function. In a more general context, our observations may have clinical relevance in those situations in which the measurable effect of a stimulation test is modulated by receptor and/or transduction systems showing quantitative genetic variability.

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