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Estimates of Genetic and Environmental Components of Serum Isocitrate Dehydrogenase (ICDH) in Normal Twins

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Abstract. Environmental (b^2) and genetic heritability (h^2) of serum isocitrate dehydrogenase (ICDH) were estimated in 96 pairs of healthy twins according to a path analysis model. The results showed significant heritability (0.42) and a very low environmental component (based on obesity and level of habitual physical activity), suggesting that these factors do not influence ICDH serum activity.

Key words: Isocitrate dehydrogenase, Obesity, Heritability, Twins

INTRODUCTION

Isocitrate dehydrogenase (ICDH) is one of the enzymes of the Krebs citric acid cycle which converts isocitric acid to oxalsuccinic acid.

Determination of ICDH activity in serum and in other fluids and tissues have been described by several authors. It is one of the cellular enzymes widely distributed in various body tissues. Several investigators observed that diseases of the hepato-biliary system were often associated with significantly elevated serum ICDH activities which occurred in the presence of inflammatory, malignant, or necrotic liver lesions [19]. In spite of the very high concentrations in heart tissue, no ICDH serum elevations are observed following myocardial infarctions [1].

In a previous investigation, genetic components of serum creatine-kinase (CK) and pyruvate-kinase (PK), estimated through path analysis, ranged from 0.49 to 0.71 in normal male and female twin pairs [17]. It was concluded that CK and PK levels were under genetic control in normal twins. Several studies reported that some factors, such as physical exercise [9], age [11], race [12,21], pregnancy [22], and seasonal variation [13], may

influence CK and PK activities in healthy subjects. However, little is known about the influence of genetic and environmental factors on ICDH activity.

In order to verify if serum ICDH levels are also under genetic control, the heritability and the environmental component were estimated through a path analysis model, in a sample of normal twin pairs who had been previously studied for CK and PK [17].

SUBJECTS AND METHODS

Ninety-six pairs of healthy twins (63 MZ and 33 DZ) with a mean age of 22.4 years ± 8.5 were studied. Determination of twin zygosity was based on six serum genetic marker systems and fifteen dermatoglyphic variables [3].

Serum ICDH activity (Sigma Units) was measured with Sigma Kits, according to a colorimetric procedure [18], that was a modification of King [8] methodology. Normal values ranged from 40 to 360 US/ml.

The genetic and environmental effects on ICDH were estimated through path analysis (Figure) according to the model described in Colletto et al [4,5]. To estimate the effect of the twins family environment [14-16], indexes were created through regression of the enzymes on obesity and level of habitual physical activity.

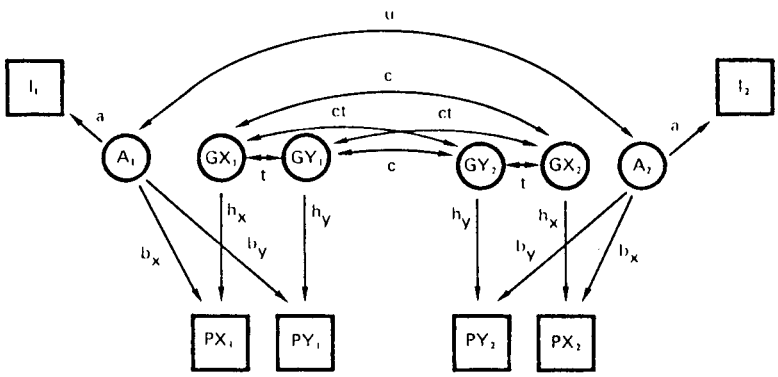

Figure. Path diagram for genetic and environment components of physiological traits in twins. For MZ twins c = 1 and DZ for twins c = ½. The subscripts 1 and 2 denote members of the twin pair. X = CK or PK and Y = ICDH.

The ratio weight/height^2 (body mass index, BMI, or Quetelet index) used as a measure of obesity [7,10,16] has been already used in reports from other investigators to create an environmental index [15,16]. Although, according to Stunkard et al [20], BMI has a high heritability, other authors suggested that this indicator of obesity is unlikely to have a sizable genetic basis [7,10] and Garn et al [6] concluded that fat people have fat relatives whether they are genetically related or not.

The level of habitual physical activity, which in the study included gymnastics, jogging and any kind of sports, was quantified from 0 to 7, according to the number of days per week in which physical activity was practiced by each member of the twin pair.

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Statistical analyses were done after a log-transformation (ln) of serum enzymes to get a normal distribution. Enzyme values, obesity and exercise habits were adjusted for sex and age (age^2, age^3, sex × age, sex × age^2 and sex × age^3) through regression analysis.

Since the present path analysis model estimates the genetic and environmental components of one trait concomitantly with another one, two estimates of ICDH were done, one with PK and the other with CK levels. This path model also allows to estimate the correlation between enzyme genotypes (ICDH/PK and ICDH/CK).

The path coefficients that were used are: a = effect of indexed environmental components on the index; b_x = effect of indexed environmental components on phenotype of CK or PK; b_y = effect of indexed environmental components on ICDH phenotype; h_x = effect of genotype on phenotype of CK or PK; h_y = effect of genotype on ICDH phenotype; u = correlation among indexed environmental components of the twin pair; t = correlation between genotype x and genotype of ICDH (Fig. 1).

Following the method described by Rao et al [14-16], the estimates of the path coefficients can be obtained by taking the log likelihood as $\ln L = -\chi^2/2 + \text{constant}$;

$$\chi^2 = \sum_{i=1}^{12} n_i \, (z_i - \bar{z}_i)^2$$

where z, and $\bar{z}$, are the observed and expected z transforms of the 12 correlations described in Table 1, and n_i, is the sample size for i^{th} relationship. Parameters are estimated by maximizing in L, and the residual χ^2 is used to test hypothesis. Four hypotheses were tested, including the general model and three others, where parameters b, h and t were fixed to zero (null hypothesis) in order to provide the residual χ^2. The general model, which estimates 6 parameters, since u was fixed to 1, was tested by residual χ^2 with 12 − 6 = 6 df.

RESULTS

The means of ICDH values of MZ (167.62 US ± 58.28) and DZ (159.76 US ± 61.83) twins did not differ according to the t′-test ($t' = 0.38$, $P > 0.50$) described by Christian and Norton [2]. The ratio between total variances of ICDH activity in MZ and DZ was not statistically significant ($F = 1.34$, $P = 0.20$).

Table 1 shows the observed interclass and intraclass correlations related to the path model. Intraclass correlations of ICDH for MZ and DZ twins were 0.456 and 0.063, respectively.

As seen in Table 2, both h^2 estimates, in the general model, showed the same value (0.42) and were statistically significant ($\chi^2 = 19.08 - 3.51 = 15.57^a$; $\chi 2 = 20.70 - 4.84 = 15.86^b$; $P < 0.001$; df = 2) when tested under hypothesis III (h = t = 0). The environmental effects (b^2) were very low (0.01) for both estimates, and not statistically significant (hypothesis II). The correlation (t) between ICDH/PK genotypes was 0.31 and between ICDH/CK 0.37. Both were statistically significant, as shown by residual $\chi^2 = 8.29$ (with PK) and 8.99 (with CK), in hypothesis IV ($P < 0.05$; df = 1).

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Table 1 - Expected and observed correlations in MZ and DZ twins, according to the path model

Variables	Correlations		
	Expected	Observed	
		With PK	With CK
(I_1, PX_1), (I_2, PX_2)	ab_x	0.053	0.099
(I_1, PY_1), (I_2, PY_2)	ab_y	0.092	0.078
(I_1, PX_2) (I_2, PX_1)	aub_x	0.002	0.169
(I_1, PY_2), $)I_2, PY_1)$	aub_y	0.063	0.049
$(7_1, I_2)$[a]	a^2u	0.367	0.394
(PX_1, PY_1), (PX_2, PY_2)	$b_xb_y + h_xh_yt$	0.160	0.178
(PX_1, PX_2)[a]	$ub_x^2 + h_x^2$ (MZ)	0.646	0.594
(PX_1, PX_2)[a]	$ub_x^2 + \frac{1}{2}h_x^2$ (DZ)	0.523	0.358
(PY_1, PY_2)[a]	$ub_y^2 + h_y^2$ (MZ)	0.456	0.456
(PY_1, PY_2)[a]	$ub_y^2 + \frac{1}{2}h_y^2$ (DZ)	0.063	0.063
(PX_1, PY_2), (PX_2, PY_1)	$ub_xb_y + h_xh_yt$ (MZ)	0.198	0.254
(PX_1, pY_2), (PX_2, PY_1)	$ub_xb_y + \frac{1}{2}h_xh_yt$ (DZ)	0.014	0.007

[a] Intraclass correlation.
I = Environmental index; PX = Phenotype of CK or PK; PY = Phenotype of ICDH; 1 and 2 = members of the twin pair.

Table 2 - Tests of hypothesis and estimates of parameters for ICDH

Hypothesis	χ^2	df	h^2	b^2	t
I. General[a]	3.51	6	0.42	0.01	0.31
General[b]	2.70	6	0.42	0.01	0.37
II. $b = 0$[a]	5.68	7	0.43	0	0.32
$b = 0$[b]	4.07	7	0.43	0	0.41
III. $h = t = 0$[a]	19.08	8	0	0.30	0
$h = t = 0$[b]	20.70	8	0	0.17	0
IV. $t = 0$[a]	11.80	7	0.40	0.03	0
$t = 0$[b]	11.69	7	0.38	0.04	0

[a] x = PK, y = ICDH
[b] x = CK, y = ICDH

DISCUSSION

Previous studies with several serum enzymes [5,17] such as lactate dehydrogenase, alkaline phosphatase, glutamic oxaloacetic transaminase, serum CK and PK, showed a significant genetic heritability ($h^2 = 0.595$, 0.813, 0.687, 0.490 and 0.710, respectively).

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According to Rao [16] the desirable strategy is to include only relevant environmental variables when creating an environmental index and, therefore, the more informative variables are those with high correlation with the phenotype. However, the relevant variables to derive such informative indexes are often not available, or the genetic basis of some of these variables is unknown. Level of habitual physical activity was chosen as one of the variables since it shows some influence on other enzyme activities, such as CK [9]. However, as shown in Table 2, environmental effects on ICDH, measured through obesity and exercise habits were only 0.01, suggesting that both variables had no influence on serum ICDH levels. Although it is expected that serum ICDH activity is also influenced by other environmental factors, we did not have available other variables that could be used to estimate such an influence.

Results from the present study revealed that genetic components are important for the variation of ICDH activity. In fact, ICDH heritability estimated through $h^2 = 2(r_{MZ} - r_{DZ})$, gives a value of 0.79, suggesting again that this enzyme is under a genetic control.

On the other hand, the genetic correlations between ICDH and PK or CK (Table 2) suggest that common genetic mechanism may influence the variation of these enzyme activities.

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