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Genetic and Environmental Determinants of 17 Serum Biochemical Traits in Brazilian Twins

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The genetic and environmental effects on the levels of 17 serum biochemical quantitative traits (calcium, phosphorus, glucose, urea nitrogen, uric acid, total protein, albumin, total bilirubin, alkaline phosphatase, lactate dehydrogenase (LDH), glutamic-oxaloacetic transaminase (SGOT), total lipid, cholesterol, triglyceride, α-lipoprotein, pre-β-lipoprotein and β-lipoprotein) were estimated in 105 pairs of healthy twins of both sexes (57 MZ and 48 DZ) by path analysis. The genotype effect h^2 was significant for all traits ($P < 0.001$) and its value extended from 0.52 (α-lipoprotein) to 0.81 (alkaline phosphatase), whereas environmental effect $\bar{b}^2$ was significant ($P < 0.05$) in only 10 traits of the 17 analyzed, with the maximum value of 0.13 (cholesterol). Correlations between genotypes of paired traits were estimated and, of 136 values, 47 were significant at the 5% levels, thus indicating partial and common genetic mechanisms.

Key words: Path analysis, Serum components, Heritability, Quantitative genetics

INTRODUCTION

The health of an individual may be judged through serum levels of some clinically important chemical traits, mainly when normal ranges are properly evaluated. It is thus important to understand which factors influence the fasting blood levels of the various substances. The purpose of this paper is to estimate the environmental and genetic determinants of some blood values in normal subjects.

Seventeen biochemical quantitative traits of blood serum were studied: calcium, phosphorus, glucose, urea nitrogen (BUN), uric acid, total protein, albumin, total bilirubin, alkaline phosphatase, lactate dehydrogenase (LDH), glutamic-oxaloacetic transaminase (SGOT), total lipid, cholesterol, triglyceride, α-lipoprotein, pre-β-lipoprotein, and β-

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lipoprotein. In a previous paper [3], the genetic and environmental determinants of the last six traits were studied in detail. The same methodology is employed here to estimate the relative contribution of genetic and environmental causes as well as the interactions between the genetic components of these quantitative traits.

SUBJECTS AND METHODS

One hundred and five pairs of healthy twins of both sexes (57 monozygous, MZ, and 48 dizygous, DZ), were studied and each pair was individually interviewed for medical examination. The blood samples were drawn after overnight fasting. Seven genetic marker systems were used to establish zygosity: ABO, Rh (C. D. E. c, e), MNSs, Duffy, P, Kell, and serum haptoglobins. Four dermatoglyphic traits were also utilized for this purpose: a-b, A′-d and total ridge counts (TRC) and atd angle [3].

The biochemical values were determined in an SMA 12/60 system (Technicon Instrument Corp.) and the lipoprotein fractions were analyzed by cellulose acetate electrophoresis [2].

The genetic and environmental effects were estimated by path analysis. This method was primarily described by Wright in 1921 [13] and further developed by Morton and Rao [9, 10] in order to improve inference tests in the analysis of family resemblance. The employed model is a modification of that presented by Rao et al [11].

The data of all traits studied were previously adjusted for age and sex, by covariance, previous to standardization. The path model used is diagrammed in the Figure and the six variables are: I = environmental index; P_x = phenotype of trait X; P_y = phenotype of trait Y; G_x = genotype of trait X; G_y = genotype of trait Y; A = indexed environment with index I. The path coefficients estimated in the general model are: b_x = effect of indexed environment on phenotype X; b_y = effect of indexed environment of phenotype Y; h_x = effect of genotype on phenotype X; h_y = effect of genotype on phenotype Y; a = effect of indexed environment on the index; u = correlation between indexed environments of the twin pair; t = correlation between genotypes (G_x and G_y).

The environmental indices (I) were calculated for all 136 possible pair combinations of traits from the 17 studied traits. These indices were created by the regression of the principal component of two variables on obesity and red blood cell count, adjusted for sex and age.

The parameters of path coefficients were estimated for all 136 combinations and, in some cases, numerical problems occurred during estimation, requiring that one parameter should be fixed while the others were estimated.

Most of the variables presented a skewed distribution that became normal after some numerical transformations. Thus, the square root transformation was taken for bilirubin and logarithm transformation for the other traits, except calcium, phosphorus, total protein, and albumin, which did not require any transformation.

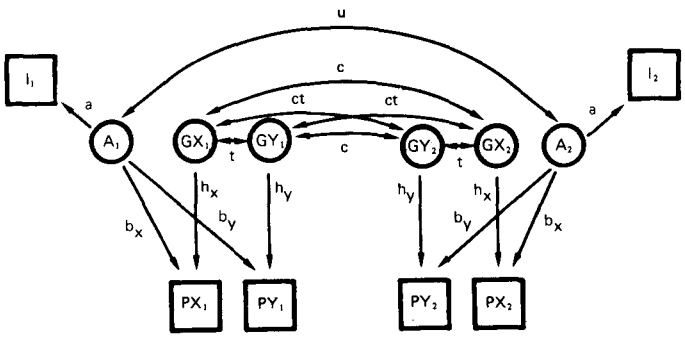

Figure. Path diagram for genetic and environmental inheritance of physiological traits in twins. For MZ twins c = 1 and for DZ twins c = 1/2. The subscripts 1 and 2 denote members of the twin pair. See text for definition of symbols. From Colletto et al [3], with permission.

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TABLE 1. Descriptive Statistics of the Studied Variables

	N		$\bar{x}$		SD		SD_a[a]		r_i[b]	
Variable (x)	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ
Calcium	56	47	9.587	9.561	0.515	0.474	0.496	0.418	0.598	0.542
Phosphorous	57	46	4.157	3.995	0.528	0.660	0.526	0.575	0.545	0.491
Glucose (ln x)	55	43	4.361	4.366	0.167	0.162	0.163	0.157	0.756	0.665
Nitrogen (ln x)	57	47	2.638	2.615	0.263	0.262	0.244	0.249	0.544	0.462
Uric acid (ln x)	56	47	1.618	1.612	0.213	0.192	0.159	0.178	0.740	0.541
Total protein	57	46	7.464	7.336	0.430	0.412	0.427	0.408	0.709	0.651
Albumin	57	47	4.837	4.625	0.479	0.396	0.463	0.362	0.787	0.637
Bilirubin (square root x)	55	47	0.848	0.803	0.163	0.132	0.152	0.131	0.789	0.523
Alkaline phosphatase (ln x)	55	45	4.147	4.165	0.352	0.380	0.303	0.291	0.802	0.631
Lactic dehydrogenase (ln x)	56	47	5.193	5.162	0.173	0.210	0.167	0.183	0.592	0.383
Glutamic-oxaloacetic transaminase (ln x)	56	46	2.891	2.864	0.454	0.413	0.423	0.377	0.692	0.288
Lipid (ln x)	57	44	6.542	6.493	0.189	0.211	0.177	0.200	0.695	0.525
Cholesterol (ln x)	57	46	5.171	5.113	0.157	0.185	0.146	0.165	0.816	0.504
Triglyceride (ln x)	57	45	4.347	4.352	0.582	0.577	0.515	0.444	0.804	0.475
β-Lipoprotein (ln x)	52	41	5.579	5.507	0.293	0.274	0.284	0.252	0.694	0.511
Pre-β-lipoprotein (ln x)	52	41	5.112	5.144	0.425	0.542	0.372	0.399	0.686	0.465
α-Lipoprotein (ln x)	57	45	5.465	5.535	0.249	0.254	0.225	0.253	0.537	0.343

[a]SD_a = only the standard deviation of the sex-age adjusted variable is shown, since the means are obviously identical.

[b]r_i = intraclass correlation.

TABLE 2. Estimates of Environmental Determinants of Serum Biochemical Values

	Environment		
Biochemical traits	$\bar{b}$ (range)	$\bar{b}^2 \pm$ SD	$\chi^2_{(2)}$ (range)
Calcium	0.202 (0.04–0.25)	0.043 ± 0.016	0.48–13.21**
Phosphorus	0.047 (0.02–0.07)	0.002 ± 0.001	0.42– 3.00
Glucose	0.235 (0.19–0.30)	0.054 ± 0.014	10.09–19.93***
Nitrogen (BUN)	0.195 (0.13–0.23)	0.038 ± 0.010	7.53–12.90**
Uric acid	0.314 (0.22–0.33)	0.095 ± 0.017	17.58–34.01***
Total protein	0.118 (0.03–0.21)	0.016 ± 0.010	1.47–8.85
Albumin	0.193 (0.10–0.24)	0.039 ± 0.014	3.05–13.17*
Bilirubin	0.107 (0.00–0.18)	0.014 ± 0.011	1.94–4.37
Alkaline phosphatase	0.030 (0.01–0.05)	0.001 ± 0.001	0.07– 2.66
Lactic dehydrogenase (LDH)	0.127 (0.07–0.18)	0.017 ± 0.008	1.82–7.01
Glutamic-oxaloacetic transaminase (SGOT)	0.066 (0.00–0.12)	0.003 ± 0.004	0.22–1.49
Lipid	0.293 (0.11–0.34)	0.090 ± 0.026	3.71–25.73***
Cholesterol	0.358 (0.16–0.42)	0.133 ± 0.039	25.25–41.38***
Triglyceride	0.087 (0.00–0.14)	0.009 ± 0.006	1.28–4.41
β-Lipoprotein	0.223 (0.06–0.28)	0.052 ± 0.019	0.88–16.48**
Pre-β-lipoprotein	0.187 (0.09–0.21)	0.035 ± 0.009	3.07–10.67*
α-Lipoprotein	0.180 (0.06–0.23)	0.035 ± 0.017	0.86–12.77**

Significance for b (2 df) is indicated as *, **, and *** for 5%, 1%, and 0.1%, respectively, based on the mean value of the χ^2.

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RESULTS

Descriptive statistics of the studied variables are presented in Table 1. For each variable, average estimates ($\bar{b}$) of environmental determinants have been calculated (Table 2). These averages (weighted by the amount of information) were obtained from all the estimates of b (sixteen in each case) for each trait. The range of b values for each trait is lower than one standard deviation in most of the cases. The values of $\bar{b}^2$ were calculated as an average from all b estimates. The residual χ^2 (two degree of freedom) were obtained by fixing b as 0 and a as 1. The significance level of each χ^2 (assigned with *) was based on the mean value of χ^2 for each trait. Among these values, sometimes the smallest χ^2 was not significant; however, if there is an asterisk, this indicates that all other χ^2 values were significant.

The weighted averages ($\bar{h}$) of the h estimates are indicated in Table 3. The ranges for h estimates were smaller than those observed for b estimates. The values for $\bar{h}^2$ were calculated as an average from all h estimates (sixteen in each case) for that parameter.

The genotype effect $\bar{h}^2$ is significant for all studied traits ($P < 0.001$) and its value extends from 0.520 (α-lipoprotein) to 0.813 (alkaline phosphatase). The environmental effect $\bar{b}^2$, however, is significant ($P < 0.05$) in only 10 of the 17 traits, and its maximum value is 0.133 (for cholesterol).

Table 4 presents estimates of the correlations between genotypes of paired traits. As can be observed, calcium shows a negative correlation with all lipid variables, although not significant for two of them, and a positive correlation with glucose, nitrogen, total protein, and albumin. The enzymes LDH, alkaline phosphatase, and SGOT show positive values for all three estimates, suggesting that, in part, common mechanisms might be responsible for the observed genetic effects.

TABLE 3. Estimate of Genetic Determinants of Serum Biochemical Values

Biochemical trait	Heritability		
	$\bar{h}$ (range)	$\bar{h}^2 \pm$ SD	$\chi^2_{(2)}$ (range)
Calcium	0.785 (0.77–0.80)	0.617 ± 0.014	19.80–48.88
Phosphorus	0.767 (0.76–0.77)	0.588 ± 0.001	30.27–37.08
Glucose	0.857 (0.84–0.87)	0.734 ± 0.015	53.16–70.19
Nitrogen (BUN)	0.740 (0.73–0.75)	0.546 ± 0.011	23.60–33.51
Uric acid	0.826 (0.81–0.84)	0.680 ± 0.016	29.01–50.02
Total protein	0.856 (0.84–0.86)	0.733 ± 0.010	17.40–65.17
Albumin	0.880 (0.86–0.89)	0.773 ± 0.015	23.83–83.65
Bilirubin	0.888 (0.88–0.89)	0.787 ± 0.008	35.31–73.06
Alkaline phosphatase	0.904 (0.90–0.91)	0.813 ± 0.007	27.50–88.73
Lactic dehydrogenase (LDH)	0.770 (0.76–0.78)	0.595 ± 0.010	23.88–32.97
Glutamic-oxaloacetic transaminase (SGOT)	0.826 (0.82–0.83)	0.687 ± 0.006	39.38–45.64
Lipid	0.799 (0.78–0.81)	0.635 ± 0.026	19.51–47.37
Cholesterol	0.837 (0.82–0.89)	0.701 ± 0.036	23.66–49.95
Triglyceride	0.896 (0.89–0.90)	0.804 ± 0.009	69.91–86.69
β-Lipoprotein	0.813 (0.80–0.84)	0.664 ± 0.021	24.31–48.05
Pre-β-lipoprotein	0.814 (0.81–0.83)	0.666 ± 0.012	37.93–56.00
α-Lipoprotein	0.723 (0.70–0.74)	0.520 ± 0.019	18.61–38.82

Significance was estimated as residual χ^2 (2 df), fixing the trait in study as zero, and all values are highly significant ($P < 0.001$).

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TABLE 4. Correlation Between Genetic Components of Serum Biochemical Traits

Biochemical traits	Calc	Phos	Gluc	BUN	Uric Acid	Prot	Albu	Bili	Alka Phos	LDH	SGOT	Lip	Chol	Trig	β-lip	Pre-β-Lip
Calcium																
Phosphorus	−0.12															
Glucose	0.28***	−0.22*														
Nitrogen (BUN)	0.22*	0.19	0.00													
Uric acid	−0.08	0.06	−0.01	0.38***												
Total protein	0.53***	−0.15	0.14	0.14	0.02											
Albumin	0.36***	−0.04	0.22***	0.07	−0.08	0.45***										
Bilirubin	0.02	0.08	0.16	−0.03	0.20**	0.05	0.19**									
Alkaline phosphatase	0.08	0.02	0.11	−0.10	0.00	0.07	−0.06	−0.23**								
Lactic dehydrogenase (LDH)	0.04	0.03	0.21*	−0.06	0.03	0.23*	−0.10	−0.12	0.34***							
Glutamic-oxaloacetic transaminase (SGOT)	−0.07	−0.08	0.07	−0.02	0.12	0.28***	0.09	−0.04	0.27***	0.43***						
Lipid	−0.29**	−0.07	−0.08	0.06	0.07	0.02	−0.15	0.10	−0.17	0.03	−0.04					
Cholesterol	−0.21*	0.05	−0.01	0.11	0.02	0.08	0.12	0.06	0.07	0.04	0.15	0.78***				
Triglyceride	−0.15	0.00	−0.23**	0.05	0.41***	0.05	−0.19***	−0.04	0.02	−0.03	0.22**	0.39***	0.24**			
β-Lipoprotein	−0.24*	0.10	−0.04	0.38***	0.10	0.03	0.00	0.21*	−0.15	−0.11	0.07	0.99***	0.76***	0.22**		
Pre-β-lipoprotein	−0.32***	−0.01	−0.24**	−0.01	0.47***	−0.06	−0.19*	0.09	−0.13	0.00	0.02	0.65***	0.28***	0.83***	0.40***	
α-Lipoprotein	−0.12	−0.21*	0.05	−0.14	−0.33***	−0.01	0.02	−0.08	0.09	−0.07	−0.15	0.55***	0.41***	−0.25**	0.19***	−0.26*

Significance was estimated as the residual χ^2, fixing t as zero (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

Triglyceride and pre-β-lipoprotein show negative correlations with glucose and albumin; henceforth, as expected, glucose shows a positive correlation with albumin. Triglyceride and pre-β-lipoprotein also show a positive correlation with uric acid. These observations indicate that genetic mechanisms responsible for the levels of triglyceride and pre-β-lipoprotein are partially shared.

Some of the significant correlations observed were to be expected since one of the chemical parameters of the pair is contained in the other, such as: albumin/total protein; total lipid/lipoprotein; triglyceride/pre-β-lipoprotein.

All these facts taken together, therefore, indicate that the correlations observed between the estimates of genetic determinants were rather consistent.

DISCUSSION

A striking predominance of relatively large genetic components was observed for the 17 serum biochemical traits. Except for cholesterol, the environmental effect ($\bar{b}^2$) was smaller than 0.10. It has to be kept in mind, however, that twin studies tend to overestimate the genetic component. First of all, the common environmental effect is rather difficult to measure. Second, the sampling method of voluntary participation also tends to increase phenotypic similarity [8]. Furthermore, the overnight fasting (12–14 hr), prior to blood sampling, would also minimize short-time influences from diet, thus reducing the environmental component.

The choice of body weight (obesity) and RBC count as composing the environmental indices poses some problems and requires caution in the interpretation of the meaning of $\bar{b}^2$ values. In spite of that, the extreme low value observed for triglyceride constitutes a kind of a surprise. One would expect a higher $\bar{b}^2$ value for this chemical constituent, surely related to fat accumulation and body weight.

It is important to emphasize that our estimates express the determinants of serum biochemical parameters observed in fasting normal young adults. The behavior of these serum values when the subject is submitted to the usual variations of normal metabolic activity might not follow the same pattern. Environmental component might increase when a larger diet differential is introduced. In spite of this rationale, Havlik et al [5] found an h^2 value of 0.88 for blood glucose 1 hr after a 50-gm oral load, an estimate higher than our fasting $\bar{h}^2$ for glucose, 0.73. Thus, it seems clear that only an experimental approach will make possible a correct evaluation of the genetic and environmental influence on the levels of the biochemical components under various metabolic conditions. On the basis of the present findings, however, it is reasonable to assume, for instance, that diet differences will affect cholesterol serum values much more than values for triglyceride, since fasting $\bar{b}^2$ was 0.133 ($P < 0.001$) for the former and only 0.009 ($P > 0.05$) for the latter, although the evidence of a rather low repeatability of triglyceride levels [12] does not support this inference.

Except for lipidic traits, which have been discussed previously [3], few reports are available concerning the inheritance of the serum components studied here. In their twin study, Havlik et al [5] obtained similar h^2 values for three substances (bilirubin = 0.48; uric acid = 0.52; nitrogen BUN = 0.56) in spite of the use of the traditional formula for h^2. Barbosa et al [1] have recently performed a combined familial and twin study, including data from the literature [4, 6, 7], through path analysis. They have consistently found lower h^2 values for total protein and albumin, compared with the present results. As a matter of fact, their h^2 estimate for total protein was not even significantly different

from zero. This discrepancy might be due to the statistical models employed and/or to sampling procedures, including population heterogeneity.

The correlations between the genetic determinants surely present a fertile field for speculation, although it seems difficult to explain through biochemical reasoning certain observed interactions. In spite of the consistency of the correlations, it is hard to explain how part of the genetic determinant of serum calcium levels, for instance, is positively correlated with the genetic determinants for serum glucose, BUN, total protein, and albumin, and negatively correlated with all the lipid variables evaluated. These findings may stimulate the search for possible common biochemical mechanisms of a genetic nature. However, the possibility of a methodological artifact cannot be discarded until additional research adds new evidence.

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