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QUANTITATIVE DIFFERENCES BETWEEN HUMAN RED CELL ESTERASE D PHENOTYPES

M.N. Klautau-Guimarães[1], M.A. Mestriner[1] and H. Krieger[2]

ABSTRACT

Red cell ESD enzyme activity was determined in 125 ESD1 phenotype individuals, 69 ESD2-1 individuals and 9 ESD2 individuals. Mean activity for the three groups was 265.04, 230.35 and 161.66 units, respectively, a unit being defined as the amount of enzyme capable of hydrolyzing 10^{-7} mol 4-methyl-umbelliferyl acetate/hour/g hemoglobin (Hb).

The distribution of enzyme activity in the sample appeared to be unimodal, with significant variability among phenotypes. The activity associated with the *ESD*1* allele was estimated to be 60% higher than that associated with the *ESD**2 allele, this difference accounting for 15% of the total quantitative variation of red cell ESD in this sample.

INTRODUCTION

Erythrocytic ESD shows electrophoretic polymorphism determined by two alleles, *ESD*1* and *ESD**2 (Hopkinson *et al.*, 1973) and a large number of rare variants (see Munier *et al.,* 1988) at a locus of the long arm of chromosome 13q14 (Sparkes *et al.*, 1980). This polymorphism is commonly used in human population studies.

Preliminary quantitative studies of this enzyme by Scott and Wright (1978) showed that the proportion of catalytic activity in heterozygotes for isozymes ESD1, ESD2-1 and ESD2 was about 2:2:1. Subsequent studies using population analysis of ESD

[1] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto-USP, 14049 Ribeirão Preto, SP, Brasil. Send correspondence to M.N.K.-G.

[2] Departamento de Genética, FIOCRUZ, Av. Brasil, 4365, 20010 Rio de Janeiro, RJ, Brasil.

activity showed that the catalytic activity of the isozyme produced by allele *ESD*1* is higher than that of the isozyme produced by allele *ESD*2* (Nishigaki *et al.*, 1983; Horai and Matsunaga, 1984). Sparkes *et al.* (1979) detected the presence of the silent allele ESD*0 in heterozygotes after quantitative and qualitative characterization of ESD.

The genetic heterogeneity of ESD activity has interesting implications for the areas of physiology, biochemistry and evolution. The study of the activity/phenotype relationship may be used to determine the reasons for maintenance of this polymorphism and the disequilibrium for this system detected in certain populations (see Simões,eseses 1980).

MATERIAL AND METHODS

Samples were obtained from a population of 203 individuals including patients with paracoccidioidomycosis (South American blastomycosis). A multidisciplinary study of South American blastomycosis was carried out by Nogueira (1986) and the aim of this search was the study of genetic variability for susceptibility to this disease, including the dynamics of biochemical polymorphism. Families were ascertained from the records of the University Hospital of Londrina-Paraná, Brazil. Subjects were classified as caucasians (78.89%) blacks and mullattoes (13.07%) and japanese and amerindians (9.04%) (for more details see Nogueira, 1986).

Blood was collected into heparinized tubes and plasma was removed by centrifugation. Red cells were washed three times in 0.9% physiological saline and lysed by vortex shaking.

ESD isozymes were detected by 2% starch-0.8% agarose gel electrophoresis using 0.1 M Tris-HCl/0.01 M EDTA/0.1 M maleic anhydride/0.01 M magnesium chloride as the bridge buffer, pH 7.4. For the gel the same buffer was diluted 1:15. Staining was by the original method of Hopkinson *et al.* (1973).

The ESD activity of the samples was determined fluorimetrically in duplicate by the method of Sparkes *et al.* (1979), one enzyme unit being defined as the amount capable of hydrolyzing 10^{-7} mol 4-methyl-umbelliferyl acetate/hour/g Hb. Hemoglobin (Hb) concentration was determined by the cyanmethemoglobin method.

RESULTS

The distribution of ESD phenotypes and the estimate of ESD gene frequencies are presented in Table I. The gene frequencies obtained show that the total sample and the patient are in Hardy-Weinberg equilibrium. Since part of the sample consisted of

patients (64 individuals with confirmed paracoccidioidomycosis, PCM), all analyses were performed on the sample as a whole (patients and immediate members of their families) and on the sample minus the patients in order to avoid the interference of factors related to disease. Only the results that differed between samples are reported separately.

Table I - Phenotype distribution and gene frequencies of ESD in the total sample and in the patient sample (paracoccidioidomycosis).

		No. of individuals		
	Phenotype	Observed	Expected	Gene frequency
Total	ESD1	125	125.4	*ESD*1* - 0.786
Sample	ESD2-1	69	68.29	χ^2 - 0.017
	ESD2	09	9.29	*ESD*2* - 0.214
Patient	ESD1	42	42.05	*ESD*1* - 0.817
Sample	ESD2-1	19	18.83	χ^2 - 0.007
	ESD2	02	2.11	*ESD*2* - 0.183

Overall ESD activity distribution (Figure 1) appears to be unimodal, with the three qualitative phenotypes overlapping. The highest values were obtained for individuals with the ESD1 phenotype and the lowest for ESD2 individuals, demonstrating variability between and within phenotypes. A level of activity of more than 600 units was observed in one individual of ESD1 phenotype. This distribution is not normal, as there was significant assymetry and positive kurtosis (Table II). Log transformation of the data gave a better fit to the normal distribution for statistical analysis (NLESD).

Table II - Assymetry and kurtosis data after natural log transformation of the variable (ESD activity).

	N	Mean ± SD	Assymetry	t	Kurtosis	t
ESD activity	204	248.583 ± 72.61	1.21385	7.1296	3.65104	31.78679
NL activity	204	5.475 ± 0.2863	- 0.12971	- 0.7619	1.0224	8.90128

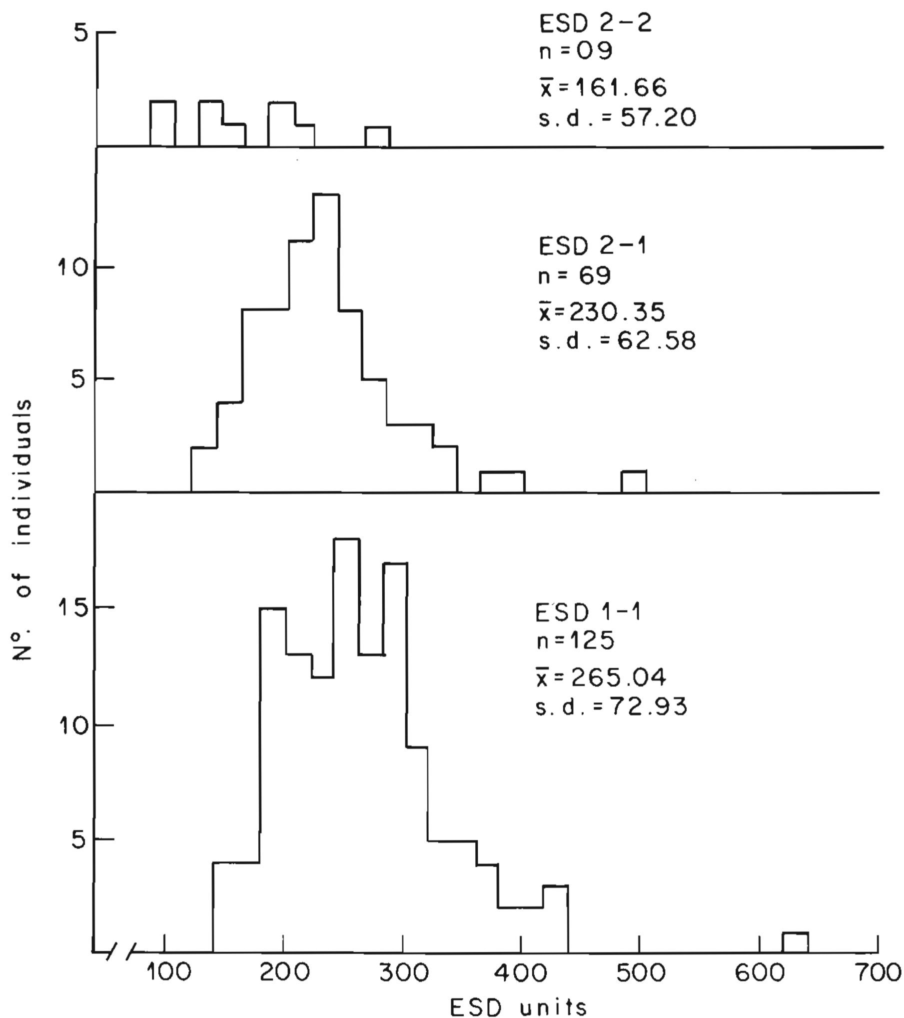

Figure 1 - Distribution of ESD activity by qualitative phenotype in the total sample. One enzyme unit was defined as the amout capable of hydrolyzing 10^{-7} mol 4-methyl-umbelliferyl acetate/hour/g Hb.

Regression and variance analyses (Table III) demonstrated a significant effect of phenotype on ESD activity ($P < 0.001$, Student t-test).

Table III - Regression analysis of ESD activity in the qualitative ESD phenotype.

NL ESD		$\bar{y}$ - 5.472 ± 0.2865	a - 5.743 ± 0.017	
Independent variable		$\bar{X}$ ± SD	b ± SEM	
ESD		1.423 ± 0.579	- 0.191 ± 0.0324	
		Analysis of variance		
Source of variation	d.f.	SS	F	r^2
Regression	1	2.4417	34.7463**	0.1486
Residue	199	13.9842		
Total	200	16.4259		

** P < 0.001.

N.L. ESD - natural log of ESD activity; ESD - qualitative esterase D.

DISCUSSION

The sample was in Hardy-Weinberg equilibrium for this system. The frequency of the *ESD*1* allele was 78.6%. When this value is compared with the frequencies obtained for other Brazilian populations (Table IV), a variation in frequency is noted, possibly due to the different degrees of racial admixture in the Brazilian population, or possibly as a random occurrence.

An interesting aspect of the population genetics of ESD is the evidence of a shift from equilibrium in the frequencies of the different phenotypes in some of the populations investigated. This shift has been detected in populations from all continents (almost 10% of the samples investigated), generally expressed as a lack of heterozygotes (Moro Furlani, 1977). The causes of this disequilibrium are still unknown and its magnitude can not be easily explained by consanguinity, preferential marriages, recent population admixture or technical errors in phenotype classification. Thus, the alternative hypotheses of selection against heterozygotes and/or the presence of a "silent" allele seems to be the most likely explanation (Simões,eseses 1980).

A silent allele was demonstrated in a study by Sparkes *et al.* (1979), who, by analysing qualitatively and quantitatively a North American family, showed segregation of the *ESD*0* allele. This segregation was also demonstrated by Koziol and Stepien (1980), and the frequency of the *ESD*0* allele was estimated as 0.115 by Marks *et al.* (1977) in an African population.

Table IV - Frequency of the *ESD*1* allele in Brazilian populations.

Location	Frequency of the *ESD*1* allele	Sample size	Reference
Londrina - Paraná	78.6%	203	Present paper
Ribeirão Preto - São Paulo	86.7%	517	Sampaio (1984)
(Mothers and newborn infants)	88.7%	517	
Curitiba - Paraná	88.0%	100	Cupli and Lourenço (1984)
(caucasians and blacks)	89.0%	390	
Porto Alegre - Rio Grande do Sul	87.0%	363	Rieger *et al.* (1988)
Bambuí - Minas Gerais	91.0%	393	Nogueira (1981)
Northeast	88.56%	2.234	Mestriner (1976)
São Paulo - São Paulo	90.5%	85	D'Almeida (1988)
Pará	91.0%	200	Schneider *et al.* (1987)
(blacks)			

It has been suggested that ESD polymorphism may be much more complex than thought thus far (see Henke *et al.*, 1986). It is interesting to note that 19 alleles have already been detected for this enzyme system (Munier *et al.*, 1988). In addition to *ESD*1* and *ESD*2*, three other alleles with polymorphic frequencies have been detected: allele *ESD*3N* in a Negrito population from the Philippines (Omoto *et al.*, 1978), allele *ESD*5* in an Italian population (Destro-Bisol *et al.*, 1986) and allele *ESD*7* in a Japanese population (Nishigaki and Itoh, 1984).

The curve for the distribution of ESD activity in the present sample was unimodal, with three phenotypes overlapping. This distribution is similar to that detected by Horai and Matsunaga (1984) in Japan, but differs from the results of Nishigaki *et al.* (1983) who detected a clearly trimodal curve, possibly due to the different methodologies used to quantify ESD activity.

The mean activity of the ESD1 phenotype was 265.04 ± 72.93 U, and the mean activity of the ESD2 phenotype was 161.66 ± 57.28 U, suggesting that the activity of the *ESD*1* allele is 60% higher than that of the *ESD*2* allele. The mean activity of the ESD2-1 phenotype was 230.35 ± 62.58 U, a value close to the calculated mean for ESD1/2 + ESD2/2 = 213.35 U when the additive gene effect is postulated as suggested by Horai and Matsunaga (1984).

Analysis of variance suggests that approximately 15% of the total quantitative ESD variation is associated with electrophoretic polymorphism of the enzyme. Thus,

activity may not be solely a function of the two alleles proposed but also of other biological and/or environmental variables. Such variables may possibly clarify the nature of intraphenotypic (non-electrophoretic) variation, which is still unknown. Two additional factors may contribute to this variation: 1) not only ESD presented activity with the substrate 4-methyl-umbelliferyl acetate; even though the assay was performed at pH 5.5 other esterases also contributed to total esterase activity; 2) other alleles may exist that were not detected by the methods used here.

The relative contribution of electrophoretic variation to total quantitative variation has also been determined in other polymorphic red cell enzyme systems (see Table V, adapted from Battistuzzi *et al.*, 1977). According to these investigators, these values (percent contribution) for a given gene depend on its degree of polymorphism and on the magnitude of the difference in activity associated with the polymorphic alleles.

Table V - Relative contribution of qualitative polymorphism to total variability of the activity of some red cell enzymes.

Enzyme system	% contribution	Reference
Acid phosphatase (ACP)	58.7	Battistuzzi *et al.* (1977)
Adenilate kinase (AK)	5.4	Battistuzzi *et al.* (1977)
Glutamate pyruvate transaminase (GPT)	38.4	Battistuzzi *et al.* (1977)
Adenosine deaminase (ADA)	7.0	Battistuzzi *et al.* (1977)
Uridine monophosphate kinase (UMPK)	10.7	Battistuzzi *et al.* (1977)
Phosphoglycomutase (PGM1)	24.0	Scacchi *et al.* (1983)
Glicose-6-phosphate dehydrogenase (G-6-PD)	7.96	Battistuzzi *et al.* (1977)
Alkaline phosphatase (ALP)	10.0	Lucarelli *et al.* (1982)
Esterase D (ESD)	15.0	Present paper

The positive correlation between allele activities and their relative frequencies is interpreted as a factor favoring the hypothesis that the common allele is not neutral (Modiano, 1976, cited by Lucarelli *et al.*, 1982). If this is true, ESD polymorphism may be transitory, leading to fixation of the *ESD*1* allele. This hypothesis may clarify the disequilibrium of the ESD system reported for some populations and should be investigated together with the hypothesis of the existence of selection against heterozygotes (Moro-Furlani, 1977), considering that the causes of disequilibrium are varied.

The electrophoretic difference between allele *ESD*1* and allele *ESD*2* reflects a difference in terms of enzyme activity unit; at present we do not know whether it is due to different catalytic activities or to different amounts of protein.

If the ESD variation occurring with 4-methyl-umbelliferyl acetate corresponds to that occurring with the natural substrate, which is unknown, these data could contribute to the understanding of disequilibrium.

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RESUMO

A atividade enzimática da ESD eritrocitária foi determinada em 125 indivíduos de fenótipo ESD1, 69 de ESD2-1 e 9 de ESD2. A atividade média desses três grupos é 265,04; 230,35 e 161,66 unidades definidas como a quantidade capaz de hidrolisar 10^{-7} moles de acetato de 4-metil-umbeliferona/h/g Hb.

A distribuição da atividade enzimática na amostra sugere ser unimodal. A variabilidade entre os fenótipos é significativa. A atividade associada ao alelo *ESD*1* é estimada ser 60% maior que a do alelo *ESD*2*. A diferença é responsável por 15% da variação quantitativa total da ESD eritrocitária nesta amostra.

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