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Genetic Study of Red Cell Esterase D Polymorphism by Ultrathin Layer Isoelectric Focusing

Distribution in the Veneto Population (Italy)*

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Summary. Human red cell Esterase D (EsD) was analyzed by isoelectric focusing (IEF) on ultrathin-layer polyacrylamide gel with a pH range of 5.0–6.0. Hemolysates were treated with Dithiothreitol to avoid loss of activity and change of the isozyme patterns by in vitro storage effects. In our sample of 951 unrelated persons from Veneto, seven different phenotypes were observed. The following allele frequencies were calculated: $EsD^1 = 0.8476$, $EsD^2 = 0.1336$, $EsD^5 = 0.0178$, and $EsD^V = 0.0010$.

Our gene frequencies have been compared to those found in other populations.

Key words: Esterase D, genetic polymorphism (EsD) – Blood groups, Esterase D

Zusammenfassung. Die Esterase D (EsD) der roten Blutkörperchen wurde mittels Isoelektrofokussierung auf einer ultradünnen Schicht von Polyacrylamidgel bei einem pH-Bereich von 5.0–6.0 analysiert. Die Hämolysate wurden mit Dithiothreitol behandelt, um Aktivitätsverlusten und einer Veränderung der Isozymmuster, bedingt durch die in-vitro-Lagerung, vorzubeugen. Bei 951 nicht verwandten Personen aus dem Veneto wurden sieben verschiedene Phänotypen beobachtet. Es wurden folgende Genfrequenzen berechnet: $EsD^1 = 0.8476$, $EsD^2 = 0.1336$, $EsD^5 = 0.0178$ und $EsD^V = 0.0010$.

Die von uns entdeckten Genfrequenzen wurden mit den in anderen Bevölkerungsgruppen gefundenen Frequenzen verglichen.

Schlüsselwörter: Esterase D (EsD), genetischer Polymorphismus (EsD) – Blutgruppen, Esterase D

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Introduction

The polymorphism of Esterase D (EsD; EC3.1.1.1) was discovered by Hopkinson et al. [1], who showed that the three common phenotypes were governed by two alleles EsD^1 and EsD^2 at an autosomal locus. The original technique was based on starch gel electrophoresis; later on several other gel media have been shown to work as well [2–4]. In the last few years rare phenotypes have been described disclosing the existence of three rare alleles, EsD^3, EsD^4, and EsD^6 [5–7]. The evidence for the subdivision of the EsD^2 allele was first reported by Martin [8]. This allele, designated EsD^5, was detected by agarose gel electrophoresis, but using only this technique it was sometimes difficult to distinguish between the products of the EsD^2 and EsD^5 alleles.

The adoption of isoelectric focusing (IEF) has resulted in a simpler and more definite separation of the products of both alleles.

Using the IEF technique on ultrathin-layer polyacrylamide gel with a pH range of 5.0–6.0 we observed the EsD 1, EsD 2, EsD 2–1, EsD 5–1, EsD 5–2, common phenotypes and two variant phenotypes designated EsD 1–V and EsD 2–V, produced by the combination of the common alleles EsD^1 and EsD^2 with a rare allele EsD^V.

In this paper we report the distribution of EsD phenotypes and their frequencies in the Veneto population.

Materials and Methods

Fresh blood samples from 951 unrelated donors were provided by the Transfusion Center of the Civil Hospital of Padua. The hemolysates were prepared from packed red cells; diluted 1:1 with 0.05 *M* Dithiothreitol, by freezing and thawing. The EsD isozyme determination was

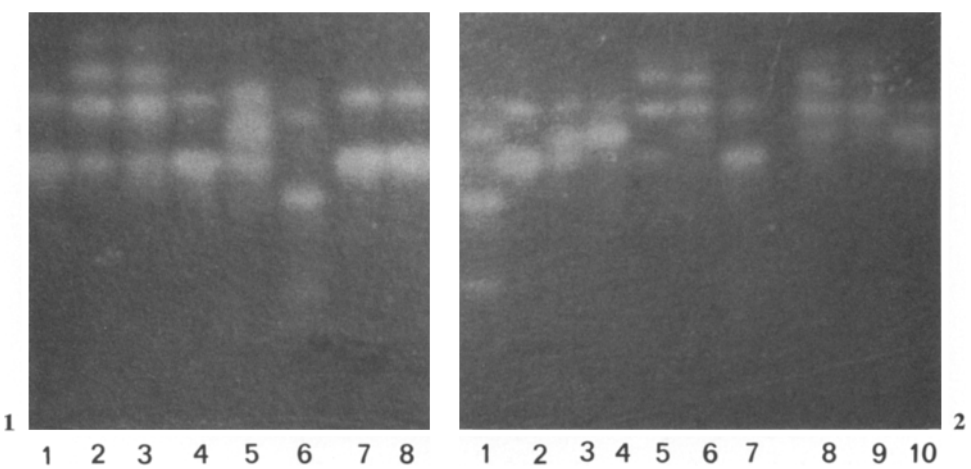

Fig. 1. EsD banding patterns after isoelectric focusing on polyacrylamide gel. From *left* to *right:* 1 (1), 2 (5/1), 3 (5/1), 4 (1), 5 (2/1), 6 (2/V), 7 (1), 8 (1)

Fig. 2. EsD banding patterns after isoelectric focusing on polyacrylamide gel. From *left* to *right:* 1 (2/V), 2 (1), 3 (2/1), 4 (2), 5 (5/1), 6 (5/2), 7 (1), 8 (5/2), 9 (5/2), 10 (2)

Table 1. The distribution of EsD phenotypes and gene frequencies in the Veneto population

EsD phenotypes	No. observed	Observed (%)	No. expected
1–1	686	72.1346	683.2228
2–1	211	22.1872	215.3811
2–2	18	1.8927	16.9743
5–5	0	0.0000	0.3012
5–1	28	2.9443	28.6958
5–2	6	0.6309	4.5229
1–V 1 2–V 1	2	0.2103	1.9019
	951	100.0000	951.0000

Gene frequencies: $EsD^1 = 0.8476$; $EsD^2 = 0.1336$; $EsD^5 = 0.0178$; $EsD^V = 0.0010$. $\chi^2 = 0.9678$; for 6 *d.f.*; $0.98 < P < 0.99$

Table 2. Comparison of EsD allele frequencies in several populations

No. of cases	Populations (authors)	EsD^1	EsD^2	EsD^5	EsD^V
1000	South-East England [9]	0.8856	0.0946	0.0198	–
711	Switzerland [10]	0.8720	0.1083	0.0197	–
384	Norway [11]	0.90	0.08	0.02	–
674	Southern Germany [12]	0.8746	0.1503	0.0185	0.0016
793	Germany [13] (Düsseldorf region)	0.897	0.088	0.015	–
1823	Japan [14]	0.628	0.364	–	0.008
3147	USA (Whites)	0.881	0.100	0.019	–
247	USA (Blacks) [15]	0.913	0.085	0.002	–
118	USA (Amerindians)	0.792	0.208	–	–
951	This study	0.8476	0.1336	0.0178	0.0010

carried out within 1 month after taking the samples. EsD typing was performed by ultrathin-layer isoelectric focusing on polyacrylamide gels (250 × 120 × 0.2 mm) on a LKB Ultraphor apparatus connected to a LKB 2297 power supply, at cool temperature. Each gel was made to a final concentration of acrylamide 5% (w/v), sucrose 12% (w/v), and ampholyte 3% (w/v) in the 5.0–6.0 pH range (Servalyt AG 5–6). After 15 min of degasation, polymerization was achieved with ammonium persulfate 0.05% (w/v). The following electrodic solutions were used: 0.25 *M* acetic acid (anolyte) and 0.25 *M* NaOH (catholyte). After 30 min of prefocusing, hemolysates were applied at 1 cm from the cathode end by means of small papers (Whatman 3 MM 7 × 4 mm), and focusing was carried out for 120 min (the paper was removed after 60 min) with the following maximal conditions 1,200 V, 15 mA 3 W, and 6°C. For the enzyme visualization a cellulose acetate membrane was soaked in a solution of 4-methylumbelliferyl acetate (5 mg dissolved in 10 drops of acetone and mixed with 10 ml of 1 *M* sodium acetate buffer, pH 5.2), blotted, and applied to the anodic area of the gel.

Results and Discussion

Figures 1 and 2 show a typical pattern of a high resolution IEF, pH range 5.0–6.0. Table 1 gives the distribution of EsD phenotypes among the Veneto population. There is a good agreement between observed and expected values assuming a Hardy-Weinberg equilibrium.

Our results were compared to those obtained by various authors with similar studies in other populations. All these gene frequencies are reported in Table 2.

The frequencies of EsD^1, EsD^2, and EsD^5 alleles found in the Veneto population show marginal differences to those carried out in other European populations and in US Whites. Differences in the distribution of EsD alleles in the various racial groups are observed: Blacks have a higher frequency for EsD^1 than Europeans; the lowest frequency for EsD^1 is found in the Japanese with 0.628.

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