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# EsD*5 Gene Frequency in Tuscany (Italy)*

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**Summary.** The distribution of the human red cell esterase D (EsD) "extended" polymorphism in a population sample from Tuscany (Italy) was studied using agarose gel isoelectric focusing. The estimated gene frequencies were: EsD*1 0.864, EsD*2 0.115, EsD*5 0.021. The EsD*5 allele frequency is very similar to those reported for other European populations. The "extension" of the EsD polymorphism may prove to be useful in paternity testing.

**Key words:** Red cell enzyme, esterase D, population genetics – Paternity testing, esterase D

**Zusammenfassung.** Die Verteilung des „ausgedehnten" Polymorphismus der Esterase-D (EsD) in menschlichen roten Blutkörperchen in der Bevölkerung der Toskana (Italien) wurde anhand der isoelektrischen Fokussierung in Agarosegel studiert. Die von uns gefundene Frequenz der Gene war: EsD*1 0,864, EsD*2 0,115, EsD*5 0,021. Die Allelenfrequenz der EsD*5 kommt denen in anderen europäischen Bevölkerungen sehr nahe. Die Anwendung des „ausgedehnten" Polymorphismus kann sich beim Vaterschaftsnachweis als nützlich erweisen.

**Schlüsselwörter:** Esterase D, Populationsgenetik – Paternitätsbegutachtung, Esterase D

## Introduction

The red cell enzyme Esterase D (EsD: E.C.3.1.1.1) polymorphism was first discovered by Hopkinson et al. (1973). Family and population studies showed that this polymorphism is determined by two common alleles, EsD*1 and EsD*2, at an autosomal locus.

Additional rare alleles have subsequently been reported: EsD*3 (Bender and Frank 1974), EsD*4 (Berg et al. 1976), EsD*6 (Radam et al. 1980), EsD*7 (Siege and Schwehn 1983), EsD*Cph (Dissing and Heriksen 1984), EsD*D

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(Henke and Basler 1984), EsD*B (Gradl et al. 1985). Nishigaki and Itoh (1984) found EsD*7 to be polymorphic in Japan, but this allele could be not identical with the independently discovered European EsD*7. A "silent" allele at the EsD locus has also been described (Marks et al. 1977; Sparkes et al. 1979; Patscheider and Dirnhofer 1979; Koziol and Stepień 1980; Gradl et al. 1985).

In 1979, a "new" common allele, EsD*5, was detected by Martin (1979a, b) by means of conventional agarose gel thinlayer electrophoresis. EsD*5 is a subdivision of EsD*2. Using the original method of Martin, a safe discrimination between 2 and 5 forms could be achieved only using an optimal technique. Better results were obtained by Martin (1981), Olaisen et al. (1981), Dykes et al. (1982) with IEF: however, their methods could not satisfactorily determine EsD 1, 2–1, and 2 phenotypes, at the same time. Consequently, for the reliable determination of the six EsD phenotypes, two procedures – one electrophoretic and one focusing technique – were necessary. Methods for the simultaneous determination of the three common EsD allozymes (1, 2, and 5) have been described by Kühnl and Spielmann (1981) and, in the last 2 years, by Budowle (1984), Bär et al. (1984), Divall (1984), and Gradl et al. (1985).

This paper reports the EsD*5 gene frequency in a population sample from Tuscany (Italy). An agarose gel isoelectric focusing (AGIF) method was used for the simultaneous detection of the EsD*1, EsD*2, and EsD*5 gene products.

## Materials and Methods

Blood specimens were collected from 700 unrelated healthy blood donors from the Blood Bank of Pisa Hospital (Tuscany, Italy). The specimens were tested within 1 week after venipuncture.

The hemolysates were prepared by freezing at −20°C and then by thawing the saline-washed red cells. They were diluted with an equal volume of 50 m*M* Dithiothreitol and incubated overnight at 4°C before the EsD type determination. Gel casting, sample application, isoelectric focusing and staining were performed in accordance with the method of Gradl et al. (1985), with minor modifications, as follows.

Gel casting: gels were casted on gelbond film (LKB) in a prewarmed cassette of two glass plates 125 × 260 mm with a plastic spacer (0.5 mm). Gel solution: 160 mg Agarose IEF from Pharmacia (0.8% w/v), 2 g Sorbitol (10% w/v), 160 mg 4-aminoethansulfonic acid (Taurine, 0.8% w/v), 250 µl Servalyt pH 4.5–5.0, and 750 µl Servalyt pH 5.0–5.5 (2% v/v) to 20 ml with destilled water. Gels were precooled overnight at 4°C. Anode was soaked with 0.25 *M* acetic acid and cathode with 0.25 *M* NaOH.

Sample application: 2 µl of DTT/hemolysate were applied 1.5 cm from the cathode with a Serva application strip.

Isoelectric focusing: prefocusing was carried out 25 min at constant power (settings: 3 W, 1,500 V, 15 mA, 5°C). Focusing was carried out 15 min at constant voltage (settings: 6 W, 400 V, 15 mA, 5°C) and 45 min at constant power (settings: 6 W, 1,500 V, 15 mA, 5°C).

Staining: cellulose acetate membranes (Biotest) were soaked in 5 ml of 0.1 *M* sodium acetate buffer (pH 7.5), containing 2 mg 4-methyl-umbelliferyl-acetate, and laid on gel surface. After incubation for 10 min at 37°C, the gel was viewed under UV light (365 nm).

## Results

The distribution of phenotypes in the population examined is shown in Table 1. The estimated gene frequencies are: EsD*1 0.864, EsD*2 0.115, EsD*5 0.021.

**Table 1.** EsD phenotype and gene frequencies in the population of Tuscany

| Phenotype | Observed | | Expected | | Gene frequencies |
|---|---|---|---|---|---|
| | *n* | (%) | *n* | (%) | |
| EsD1 | 523 | 74.71 | 522.91 | 74.70 | EsD*1 0.8643 ± 0.0091 |
| EsD2-1 | 140 | 20.00 | 139.15 | 19.88 | EsD*2 0.1150 ± 0.0085 |
| EsD5-1 | 24 | 3.43 | 25.05 | 3.58 | EsD*5 0.0207 ± 0.0038 |
| EsD2 | 8 | 1.14 | 9.26 | 1.32 | |
| EsD5-2 | 5 | 0.72 | 3.33 | 0.48 | |
| EsD5 | 0 | 0.00 | 0.30 | 0.04 | |
| Total | 700 | 100.00 | 700.00 | 100.00 | |

$\chi^2$ 1.35 for 3 *df* ($0.80 > P > 0.70$)

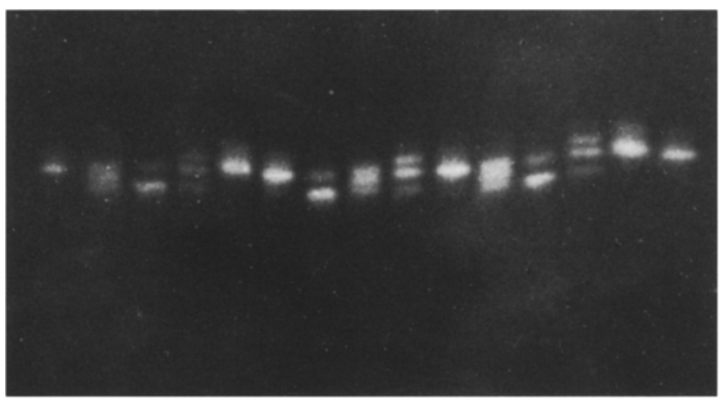

**Fig. 1.** Isoenzyme pattern of the five common EsD phenotypes. From *left* to *right*: 2, 2-1, 1, 5-1, 5-2, 2, 1, 2-1, 5-1, 2, 2-1, 1, 5-1, 5-2, 2 (anode on the *top*)

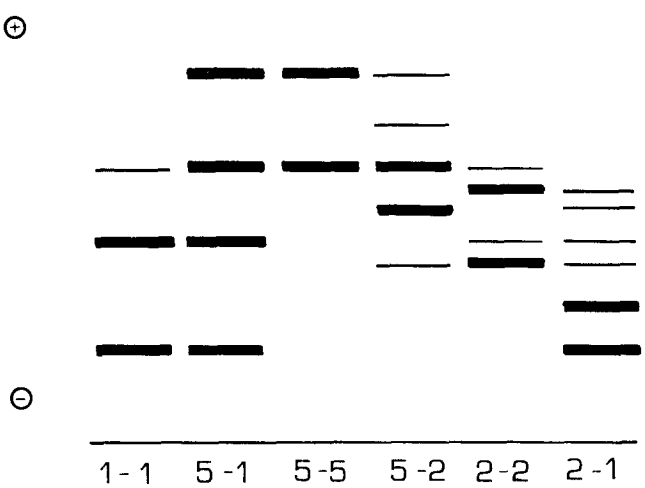


**Fig. 2.** Schematic representation of the enzyme patterns obtained from the EsD phenotypes (after Gradl et al. 1985, modified)

**Table 2.** EsD gene frequencies in some European countries

| Country | EsD*1 | EsD*2 | EsD*5 | EsD*Rare | References |
|---|---|---|---|---|---|
| Norway | 0.900 | 0.080 | 0.020 | – | Olaisen et al. (1981) |
| England | 0.885 | 0.095 | 0.020 | – | Divall (1984) |
| Germany | 0.887 | 0.099 | 0.014 | – | Martin (1979b) |
| Germany | 0.890 | 0.090 | 0.020 | – | Kühnl et al. (1981) |
| Germany | 0.897 | 0.088 | 0.015 | – | Günther et al. (1982) |
| Germany | 0.875 | 0.105 | 0.018 | 0.002 | Gradl et al. (1985) |
| Switzerland | 0.872 | 0.108 | 0.020 | – | Scherz et al. (1983) |
| Switzerland | 0.863 | 0.116 | 0.021 | – | Bär et al. (1984) |
| Italy | 0.864 | 0.115 | 0.021 | – | This paper |

**Table 3.** EsD gene frequencies in Italy

| Region | *n* | EsD*1 | EsD*2 | EsD*Rare | References |
|---|---|---|---|---|---|
| Lombardy | 549 | 0.865 | 0.135 | – | Piazza et al. (1982) |
| Veneto | 630 | 0.856 | 0.144 | – | Piazza et al. (1982) |
| Liguria | 105 | 0.862 | 0.138 | – | Piazza et al. (1982) |
| Tuscany | 500 | 0.856 | 0.143 | 0.001 | Bargagna et al. (1975) |
| Tuscany | 700 | 0.864 | 0.136 [°] | – | This paper |
| Marches | 1239 | 0.870 | 0.130 | – | Cingolani et al. (1983) |
| Latium | 499 | 0.869 | 0.131 | – | De Mercurio and De Giovanni (1980) |
| Latium | 540 | 0.840 | 0.160 | – | Piazza et al. (1982) |
| Abruzzi | 556 | 0.849 | 0.151 | – | Vecchiotti and D'Onofrio (1980) |
| Abruzzi | 439 | 0.848 | 0.152 | – | Corbo et al. (1981) |
| Southern Italy | 64 | 0.875 | 0.125 | – | Piazza et al. (1982) |
| Sicily | 228 | 0.862 | 0.136 | 0.002 | Giaccone et al. (1982) |
| Sicily | 723 | 0.864 | 0.136 | – | Bonavita and Crinò (1982) |

[°] EsD*2 + EsD*5

Figure 1 shows the pattern of the five common EsD phenotypes determined by AGIF. No subject was found to possess an EsD 5 phenotypes, whose expected frequency in the population of Tuscany is 4/10,000. Figure 2 shows a schematic pattern of the EsD phenotypes, including EsD 5. In Table 2, the frequency of the EsD*5 allele in Italy is compared with those of other European populations. Table 3 shows the frequencies of the EsD*1 and EsD*2 alleles in different regions of Italy found in previous studies.

## Discussion

The distribution of the EsD phenotypes in the population of Tuscany is in good agreement with what is to be expected on the basis of Hardy-Weinberg equil-

ibrium. The estimated frequency of the EsD*5 allele is very similar to those described in other populations of northern and central Europe. On the other hand, the EsD*1 allele, as has already been pointed out (Bargagna et al. 1975) is found with a slightly lower frequency in Italy as compared with the countries of northern Europe. However, unlike other systems (ABO, Rhesus: see Bargagna et al. 1979), there is no indication of a cline in EsD gene frequencies along the Italian peninsula.

In paternity testing, the "extension" of the EsD polymorphism increases the average chance of exclusion from 10.4% to 11.5%, based on the gene frequencies in Tuscany. It does not require the use of two distinct procedures any longer, and can thus be carried out as a routine test. When there is the suspicion of the existence of a "silent" gene, conventional electrophoresis is not sufficient, even if it is associated with the determination of the enzymatic activity. Evidence has been offered of a variation of activity between the EsD phenotypes (Nishigaki et al. 1983): therefore only by both examinations – conventional electrophoresis and quantitative enzyme assay – may a type EsD 7–1, for example, be judged to be heterozygote of a "null" allele (EsD*1/EsD*0), because of the low activity of the EsD*7 and the similar electrophoretic mobility of EsD 1 and EsD 7 (Nishigaki and Itoh 1984).

The agarose gel isoelectric focusing method described by Gradl et al. (1985) is relatively easy to handle and offers a clear discrimination between common phenotypes. It also offers the advantage of not using toxic substances, unlike polyacrylamide isoelectric focusing.

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