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Polymorphisms of the Enzyme Systems Galactose-1-Phosphate Uridyltransferase (GALT) and Esterase D (EsD) in the Province of Cádiz, Southern Spain

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ABSTRACT: Galactose-phosphate uridyltransferase (GALT) and esterase D (EsD) phenotypes were determined by isoelectric focusing in ultrathin-layer polyacrylamide gel (PAGIF) for 406 healthy subjects randomly chosen and residing in the province of Cádiz, in Southern Spain. The following gene frequencies were observed: for GALT, GALT1 = 0.952 970 3 and GALT2 = 0.047 029 71; for EsD, EsD1 = 0.895 320 2, EsD2 = 0.094 827 59, and EsD5 = 0.009 852 21.

KEYWORDS: pathology and biology, galactose-1-phosphate uridyltransferase (GALT), esterase D, genetic typing, enzymes, gene frequencies, paternity testing

In recent years, the study of the polymorphisms of the erythrocyte enzymes galactose-1-phosphate uridyltransferase (GALT) and esterase D (EsD) using isoelectric focusing (IEF) has become a routinely applied technique.

With respect to the enzyme GALT, this procedure possesses a capacity for resolution higher than that observed with other techniques of conventional electrophoresis and thus offers greater sensitivity of detection and cleaner patterns for interpretation, as well as easier analyses.

The use of IEF in the study of the EsD polymorphism has enabled a clear differentiation to be made between certain alleles common to the Caucasian population and also makes posssible identification of unusual or infrequent variations that may be present [*1–3*].

Materials and Methods

In this study, the authors have used 406 blood samples, extracted from the same number of healthy individuals resident in the province of Cádiz in Southern Spain (see Fig. 1). The individuals were chosen randomly from among those treated at outpatient departments of different hospitals for trauma. Their number was established in proportion to the population size of each municipality.

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FIG. 1—*Cádiz Province, Andalusia, Spain.*

Red cells were washed three times in physiologic saline solution. The packed cells were stored at −20°C until used for determination of GALT and EsD. Before typing, the samples were pretreated with fresh Cleland's reagent (0.05 dithiothreitol) to prevent changes that otherwise might have occurred as a result of oxidation.

The method of IEF used in the study of the markers that we observed (GALT and EsD) basically follows the norms laid down by Carracedo et al. [*1*]. The support consisted of 0.4-mm ultrathin-layer polyacrylamide gels made on 24 by 12.5 by 0.2-cm silanized glass plates. The gels were cast by capillary action. These gels contained a concentration of T [acrylamide + *N*,*N*'-methylene bisacrylamide (BIS)0/100] = 5.5% and cross-linking of C (BIS/acrylamide + BIS) = 3%. The ampholine (LKB, Broma, Sweden) concentration was 5% v/v. Polymerization of the gels was carried out by the addition to these gels of 0.2 cc of a 0.02% w/v riboflavin solution and by applying (UV) light.

The pH range used in the GALT study was 5 to 7. The electrode solutions used were, as with the other markers studied, 1*M* phosphoric acid and 1*M* ethanolamine in the anode and cathode, respectively. The samples, previously treated with dithiothreitol, were placed, using Whatmann 3MM paper (1 by 0.5 cm), 1 cm from the anode. Following this process, IEF was carried out at a constant power of 3 W, with the voltage restricted to 1.500 V and the current unlimited for 200 min. The process of development we used for this marker has been described elsewhere by Vaccaro et al. [*4*], although modifications were employed.

The pH range used in the EsD study was achieved by mixing (1:1) ampholyte at 4 to 6 pHs and 5 to 7. The samples, previously treated, were applied to the gel on Whatmann 3MM paper (1 by 0.5 cm), 1 cm from the cathode extreme. IEF was carried out at a constant power of 5 W, with the voltage restricted to 1.500 V and the current unlimited for approximately 3½ h. The developing was completed following the technique described by Hopkinson et al. [*5*].

Results and Discussion

Table 1 shows the gene frequencies observed for the different GALT alleles studied in our province, as well as the Hardy-Weinberg equilibrium recorded in the province of Cádiz for this marker.

With the GALT system, it was significant that very few differences were observed between the frequencies recorded in our province for the Gt1 allele and those observed for this variant among the greater part of European populations (Table 2) [*1,4,6–12*]. The recorded frequency of the Gt2 allele is among the lowest recorded values for this continent and is similar to the 0.040 and 0.041 gene frequencies for Galt D + Galt LA established for the English and Yugoslavian populations, respectively [*11,12*].

Likewise, the probability of exclusion of paternity established for this marker was 4.28%, a percentage lower than other values recorded in studies of similar nature in other European population groups [*7,9*].

The gene frequencies established in the present paper for the alleles EsD1, EsD2, and EsD5, identified in the EsD system, are shown in Table 3. It is evident that the population sample under study is in Hardy-Weinberg equilibrium for this marker.

The similarities between the frequencies of the alleles EsD1 and EsD2 observed in our province and those recorded for these variants in the great majority of European populations, as well as the low incidence of the allele EsD5 in our sample, are especially remarkable (Table 4) [*13–29*].

Lastly, the probability of exclusion of paternity established in the province of Cádiz for the EsD system in the sample under study was 8.96%, which is similar to that obtained in other population groups of this continent [*1,13,29*].

TABLE 1—*GALT phenotypes and gene distribution in a population sample in Cádiz Province, Southern Spain.*[a]

Phenotype	Absolute Phenotype Frequencies		Allele Frequencies
	Empirical	Theorical	
1/1	367	366.89	Gt1 = 0.952 970 3 ± 0.020 640 5
1/2	36	36.21	
2/2	1	0.89	Gt2 = 0.047 029 7 ± 0.020 640 5
Total	404	404.00	

[a] $\chi^2 = 0.013\ 960\ 11$; $df = 1$; $0.95 > P > 0.90$.

TABLE 2—*European distribution of GALT allele frequencies.*

European Population	Gt1, GALT N	Gt2			Reference
		GALT D		GALT LA	
Germany					
Southwestern	0.923		0.072		[6]
Hessen	0.929		0.071		[7]
Munich	0.937		0.060		[8]
Denmark	0.923		0.077		[9]
Spain,					
Galicia	0.930		0.070		[1]
Greece	0.942		0.058		[10]
England	0.960	0.025	0.040	0.015	[11]
Italy	0.919	0.037	0.077	0.040	[4]
Yugoslavia,					
Serbia	0.959	0.018	0.041	0.023	[12]

TABLE 3—*EsD phenotypes and gene distribution in a population sample in Cádiz Province, Southern Spain.*[a]

Phenotype	Absolute Phenotype Frequencies		Allele Frequencies
	Empirical	Theorical	
1/1	327	325.45	
1/2	65	69.94	EsD1 = 0.895 320 20 ± 0.029 776 5
1/5	8	7.16	EsD2 = 0.094 827 59 ± 0.028 497 6
2/2	6	3.65	EsD5 = 0.009 852 21 ± 0.009 601 8
2/5	0	0.76	
5/5	0	0.04	
Total	406	406.00	

[a] $\chi^2 = 2.640\ 02$; $df = 3$; $0.50 > P > 0.25$.

TABLE 4—*European distribution of EsD allele frequencies.*

Population	EsD1	EsD2	EsD5	EsDV	Reference
Denmark	0.9007	0.0099	. . .	0.0001	[13]
Norway	0.9000	0.0800	0.0200	. . .	[14]
Germany, Southern	0.8746	0.1503	0.0185	0.0016	[15]
Germany	0.8870	0.0990	0.0140	. . .	[16]
Germany	0.8900	0.0900	0.0200	. . .	[17]
Germany, Western	0.8740	0.1050	0.0180	. . .	[18]
Germany, Düsseldorf	0.8970	0.0880	0.0150	. . .	[19]
Switzerland	0.8720	0.1083	0.0197	. . .	[20]
Switzerland	0.8630	0.1160	0.0210	. . .	[21]
Italy	0.8640	0.1150	0.0210	. . .	[22]
Rome	0.8450	0.1380	0.0170	. . .	[23]
Trieste	0.8440	0.1370	0.0170	. . .	[24]
Veneto	0.8476	0.1336	0.0178	0.0010	[25]
England, Southeastern	0.8856	0.0946	0.0198	. . .	[26]
England, Oxford	0.8790	0.0860	0.0350	. . .	[27]
England					
Whites	0.8856	0.0946	0.0198	. . .	[28]
Blacks	0.9019	0.0921	0.0060	. . .	[28]
Indians	0.7548	0.2308	0.0144	. . .	[28]
Spain, Galicia	0.8744	0.1040	0.0216	. . .	[1]

In spite of the fact that the values obtained for GALT and EsD, by a priori exclusion probability and discrimination probability (DP), were not elevated (DP = 0.1670 and DP = 0.3253, respectively), we agree with other authors [*1,4,6–12,13,29*] that analyses for the above-mentioned systems should be included in the routine protocol for the biological investigation of paternity because of their ease and simplicity, high efficiency, and moderate cost. This consideration is especially true for analysis for GALT because, despite the high cost of its technical determination, the results obtained have excellent reliability. Likewise, the study of these enzymes in our province increases the list of genetic molecular markers (including Gc, Pi, MNSs, ABO, Rh, Tf, and PLG) which are commonly used in the investigation of biological paternity, a field in which we have been working in recent years.

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