ALPHA-2-HS-GLYCOPROTEIN POLYMORPHISM IN TUSCANY (ITALY)

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INTRODUCTION

The genetic polymorphysm of A2HS glycoprotein was described for the first time in 1977 by Anderson and Anderson. Using two-dimensional electrophoresis they detected three common phenotypes controlled by two codominant autosomic alleles, which they named L and N.
The study of A2HS system was remarkably improved in 1983 when isoelectric focusing followed by immunofixation was introduced independently by Cox et al. and Umetsu et al.
By means of that technique the products of the two common alleles, A2HS*1 (= L) and A2HS*2 (= N) may be recognized in all the examined populations. In Caucasian population also allele A2HS*3 (Cox et al., 1983), which is polymorphic in some geographic areas, allele A2HS*4 (Weidinger et al., 1984) and A2HS*10 (Weidinger et al., 1986) have been described. Several alleles have detected in the Orientals, including A2HS*5 (Umetsu 1984, b), which is polymorphic in Southern Japan.
A2HS locus is placed in chromosome 3 (Cox et al., 1985).
The genetic plymorphism of A2HS glycoprotein had not been investigated in Italy until now. In this paper the distribution of A2HS phenotypes in a sample of the population of Tuscany (Central Italy) has been reported.

MATERIALS AND METHODS

Sample collection: 350 samples of serum obtained from unrelated blood donors of the Hospital of Pisa have been investigated. Sera have been tested within three days from the drawing of blood. Until use sera were stored at -20 °C.
Phenotypes have been recognized using isoelectric focusing in polyacrylamide gels followed by immunofixation, according to the technique suggested by Weidinger (1986) with minor modifications.
Gel casting: isoelectric focusing was carried out with flat bed gels (250 x 115 x 0.5 mm) using glass plates treated with methacryloxypropyltrimethoxysilane; 9.0 ml solution of acrylamide (19 % w/v) + bis-acrylamide (0.6 % w/v), 1.0 ml Pharmalyte pH 4.2-4.9, 0.2 ml Pharmalyte pH 4.5-5.4, 2.4 ml Glycerol (87 %), 5.5 ml distilled water, 30 mg ACES (Serva) were mixed in a flask and deaerated for 5 min. After addition of 8 µl TEMED and 160 µl ammonium persulfate solution (3% in distilled water), gels were polymerized for 30 min. After 1 h at room

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temperature gel was stored overnight at 4 °C.
Sample application: samples of 8 µl were applied 1.5 cm from the cathode using applicator strips from Serva.
Electrode solutions: anode : 0.025 M aspartic acid + 0.025 M glutamic acid; catode: 0.1 M NaOH.
Isoelectric focusing (without prefocusing): in a Multiphor chamber (LKB 2117) at a cooling temperature of 8 °C for 3 h: 2000 V, 20 mA, 8 W for 1 h; 2000 V, 20 mA, 18 W for 2 h.
Immunoprinting: after isoelectric focusing, on gel surface were applied cellulose acetate strips (from Gelman Sci., Inc. Ann Arbor - Michigan USA) soaked in a saline diluted 1/2 anti human-2-HS-Glycoprotein antiserum (from Behering) for 3′ at room temperature. Strips were removed and washed twice in saline for 15′, stained for 5′ with nigrosine and destained for 10′ by an acetic acid 5 % solution.

RESULTS AND DISCUSSION

Distribution of the observed phenotypes is given in table 1. The good agreement between the observed and expected phenotypes, assuming Hardy-Weinberg equilibrium condition, is self- evident. The estimated allele frequencies from our population sample are: A2HS*1 = 0.7214, A2HS*2 = 0.2771, A2HS*Rare = 0.0014.
The product of the rare allele we detected in our sample is still to be classified.
Table 2 summarize the hitherto reported A2HS allele frequencies in various population. The estimated frequency of A2HS*1 allele in Central Italy appears higher than in both North European and North American countries. It seems closer to the A2HS*1 gene frequency in Oriental populations.
A2HS-glycoprotein polymorphism is suitable for being applied to the investigation of disputed paternity. In Italian population the theoretical chances of exclusion is about 16.2 % (I class exclusion: 8.2 %; II class exclusion: 8.0 %).

Table 1: Phenotype distribution and gene frequencies in a sample from Tuscany (Italy)

Phenotype	Observed n	Observed %	Expected n	Expected %	Gene Frequencies
1	181	51.71	182.16	52.05	A2HS*1 = .7214 ± .0169
2-1	142	40.57	139.96	39.99	A2HS*2 = .2771 ± .0169
2	26	7.43	26.88	7.68	A2HS*Rare = .0014 ± .0014
1-Rare °	1	0.29	0.72	0.21	
2-Rare °	0	0.00	0.28	0.08	
Rare °	0	0.00	0.00	0.00	
Total	350	100.00	350.00	100.00	

chi square: 0.0655, 1 d.f., P> 0.20
°(not included in the chi square test)

Table 2: Geographic distribution of A2HS gene frequencies

Population	n	A2HS*1	A2HS*2	A2HS*Rare	References	
Norway	52	.6000	.3900	--	Olaisen et al.	1981
Canada (Toronto)	215	.6419	.3535	.0046	Cox et al.	1986
Germany (Hessen)	197	.6550	.3400	.0050	Tarkkala Mendner	1986
Germany (Munchen)	166	.6540	.3220	.0240	Weidinger et al.	1984
Germany (South)	344	.6642	.3208	.0150	Weidinger	1986
Italy (Tuscany)	350	.7214	.2771	.0014	Present Study	
Japan (Yamagata)	2050	.7356	.2639	.0005	Umetsu et al.	1984
Japan (Izumo)	300	.7233	.2767	--	Yuasa et al.	1985
Japan (Yamaguchi)	400	.7325	.2675	--	Yuasa et al.	1985
Japan (Okinawa)	397	.7670	.2065	.0264	Yuasa et al.	1985
Carribean (Black)	71	.6901	.2606	.0493	Cox et al.	1986

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