Int J Leg Med (1991) 104:77–79

International Journal of
Legal Medicine
© Springer-Verlag 1991

Genetic polymorphism of Alpha-2-HS glycoprotein in Lombardy (Italy)

N. Cerri and F. De Ferrari

Istituto di Medicina Legale e delle Assicurazioni, Universita' degli Studi di Brescia, Piazzale Ospedale, 1, I-25125 Brescia, Italy

Received February 27, 1990 / Received in revised form May 5, 1990

Summary. α2HS-subtypes have been analysed in samples from 700 unrelated individuals from the Brescia area (Italy) by the isoelectric focusing technique and immunofixation. The observed allele frequencies were: α2HS*1 = 0.7472; α2HS*2 = 0.2507; α2HS*V = 0.0021.

Key words: Blood groups – α2HS – Glycoprotein polymorphism

Zusammenfassung: Bei 700 nicht verwandten Individuen aus Brescia (Italien) wurden die α2HS Phänotypen mittels isoelektrischer Fokussierung und anschließender Immunofixation bestimmt. Die beobachteten Allelfrequenzen waren: α2HS*1 = 0.7472; α2HS*2 = 0.2507; α2HS*V = 0.0021.

Schlüsselwörter: Blutgruppen – α2HS – Glykoproteinpolymorphismus

Introduction

Alpha-2-HS-glycoprotein (α2HS, A2HS) is a plasma glycoprotein (mol. wt. 49000), present in the serum of healthy individuals at levels between 45 and 80 mg/dl (Putnam 1984). It was first described by Heremans (1960) and later isolated by Schmidt and Burgi (1961). Alpha-2-HS glycoprotein is synthesized in the liver and deposited in mineralising human bone (Dikson et al. 1975; Triffit et al. 1976). Its biological function is not yet entirely understood: in vitro, it enhances the phagocytic function of mouse macrophages (Lewis and André 1980) and human monocytes (Lewis and André 1981); it binds to lymphocytes which have been transformed by the Epstein Barr virus and this property can be of importance to remove these cells from circulation (Lewis and André 1982, 1983). A significant decrease is observed in patients suffering from an acute inflammatory process of bacterial etiology (Lebreton et al. 1979).

The genetic polymorphism of this protein was first investigated by Anderson and Anderson in 1977 who used 2-dimensional electrophoresis. They described 2 different electrophoretic variants L and N. The presence of 2 common genetic types of α2HS (A2HS*1 = L and A2HS*2= N) has been confirmed by Umetsu et al. (1983) by means of the isoelectric focusing technique. Subsequent IEF methods have been developed with the detection of several variants which are polymorphic in some geographic areas. Cox and Andrews (1983) reported the existence of three alleles (α2HS*1, 2 and 3) in a caucasian population, in 1984 Weidinger et al. described a new variant defined as α2HS*4, while Umetsu et al. (1985) observed the A2HS*5 allele in a Japanese population. At present it is possible to distinguish a total of 15 alleles (Yuasa and Umetsu 1988). In 1985 Cox and Francke showed that the A2HSG locus is located on chromosome 3.

So far, genetic polymorphism of A2HS has not been extensively investigated and is not commonly used in paternity testing or bloodstain analysis. The aim of this study is to present a contribution to a better knowledge of the distribution of α2HS polymorphism in Italy through a population sample from the Brescia area (Lombardy).

Materials and methods

Sera from 700 healthy and unrelated blood donors, made available by the Transfusion Centre of the "Spedali Civili" of Brescia (Lombardy – Italy) have been studied. Samples were stored at −20°C before use and tested within one month.

α2HS typing was performed by thin layer isolectric focusing on polyacrylamide gel (250 × 120 × 0.5 mm), according to Weidinger (1986), with minor modifications as suggested by Giari et al. (1988).

The gel was prepared by mixing the following solutions: 9 ml acrylamide stock solution (19% w/v acrylamide and 0.6% w/v N–N' Methylene bisacrylamide), 2.4 ml glycerol (87%), 5.5 ml distilled water, 1 ml Pharmalyte pH 4.2–4.9, 0.2 ml Pharmalyte pH 4.5–5.4 and 30 mg ACES (Serva).

Polymerization was performed after the addition of 6 µl TEMED and 180 µl ammonium persulfate (3%). After 1 h at room tempera-

Offprint requests to: F. De Ferrari, Istituto di Medicina Legale e delle Assicurazioni, Spedali Civili, Piazzale Spedali, 1, I-25123 Brescia, Italy

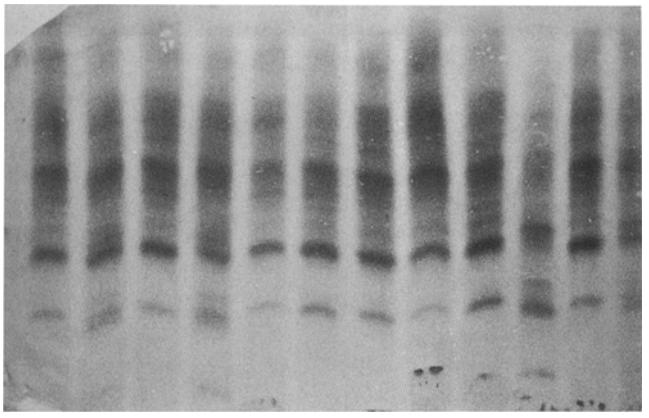

Fig. 1. Electrofocusing and immunofixation pattern of the common α2HS phenotypes. From left to right: (1) 1; (2) 2-1; (3) 1; (4) 2-1; (5–9) 1; (10) 2; (11) 1; (12) 2-1

Table 1. α2HS phenotype distribution and allele frequencies in Brescia (Lombardy – Italy)

Phenotype	Observed		Expected		Allele frequencies
	n	%	*n*	%	
1	395	56.43	390.82	55.83	α2HS* 1 = 0.7472
2-1	254	36.28	262.25	37.47	α2HS* 2 = 0.2507
2	48	6.86	44.00	6.29	α2HS*V = 0.0021
1-V	2	0.29	2.20	0.31	
2-V	1	0.14	0.73	0.10	
Total	700	100.00	700.00	100.00	

$\chi^2 = 0.7857$, 2 d.f.; $0.5 > P > 0.7$

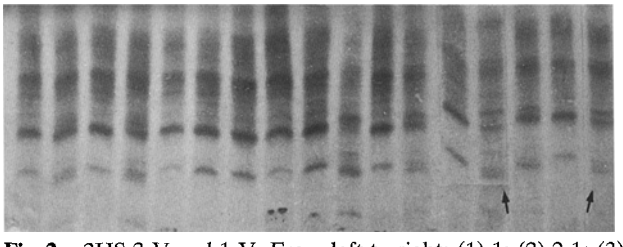

Fig. 2. α2HS 2-V and 1-V. From left to right: (1) 1; (2) 2-1; (3) 1; (4) 2-1; (5–9) 1; (10) 2-2; (11) 1; (12) 2-1; (13) 1; (14) 2-V; (15) 1; (16) 1-V

Table 2. α2HS allele frequencies in Italy

Region	*n*	α2HS*1	α2HS*2	α2HS*V	Reference
Tuscany	350	0.7214	0.2771	0.0014	Giari et al. 1988
Latium	199	0.756	0.244	–	Scacchi et al. 1989
Abrutium	106	0.788	0.212	–	Scacchi et al. 1989
Sardinia	152	0.832	0.168	–	Scacchi et al. 1989
Lombardy	700	0.7472	0.2507	0.0021	This study

ture the gel was stored overnight at 4°C. Serum samples (8 µl) were applied 1.5 cm from the cathode using applicator strips from Serva. As electrode solutions, a mixture of 0.025 *M* aspartic acid and 0.025 *M* glutamic acid was used for the anode and 0.1 *M* NaOH for the cathode.

Table 3. Geographical distribution of α2HS allele frequencies

Country	*n'*	α2HS*1	α2HS*2	α2HS*V	Reference
Norway	52	0.6000	0.3900	–	Olaison et al. 1981
England	382	0.6466	0.3469	0.0069	Westwood 1988
Germany	166	0.654	0.3220	0.0240	Weidinger et al. 1984
Germany	197	0.6550	0.3400	0.0050	Tarkkala Mender and Kühnl 1986
Germany	344	0.6642	0.3208	0.0150	Weidinger 1986
Germany	249	0.6310	0.3630	0.0060	Luckenbach et al. 1988
Germany	168	0.6250	0.3750	–	Yuasa and Umetsu 1988
France	240	0.7167	0.2750	0.0083	Robinet-Levy et al. 1988
Egypt	95	0.8579	0.1424	–	Abe et al. 1987
Libya	110	0.8364	0.1636	–	Sebetan and Heshmat 1988
Canada	215	0.6419	0.3555	0.0046	Cox et al. 1986
USA	150	0.6530	0.3470	–	Boutin et al. 1985
Japan	2050	0.7356	0.2639	0.0005	Umetsu et al. 1984
Japan	300	0.7233	0.2767	–	Yuasa et al. 1985
Japan	400	0.7325	0.2675	–	Yuasa et al. 1985
Japan	397	0.7670	0.2065	0.0264	Yuasa et al. 1985
Japan	283	0.7456	0.2544	–	Tamaki et al. 1985
Japan	256	0.7637	0.2363	–	Kishi et al. 1988
Taiwan	199	0.7286	0.2714	–	Yuasa and Umetsu 1988
Philippines	115	0.6870	0.3130	–	Umetsu et al. 1988
Nepal	140	0.7571	0.2429	–	Yuasa and Umetsu 1988

IEF was performed using an LKB Ultraphor chamber connected to the LKB 2197 Power Supply for a total of 3 h: 1 h at 2000 V, 20 mA, 8W and 2 h at 2000 V, 20 mA, 18W. The cooling unit was set at 8°C.

After seperation, α2HS phenotypes were identified by immunofixation. A cellulose acetate strip was soaked in anti-human α-2-HS-Glycoprotein (ATAB) diluted 1:3 in saline and placed on the gel surface at room temperature for 10 min. The strip was removed and washed twice in saline for 15 min, stained with nigrosine (1%) and destained in acetic acid (5%).

Results and discussion

Table 1 shows the phenotypic distribution and allele frequency values of α2HS obtained in the population sample studied from Brescia.

Three common phenotypes α2HS 1-1, α2HS 2-1, α2HS 2-2 and two rare variants were observed. These variants, defined as 1-V and 2-V, have a migration similar to the product of the allele AHSG*4, first described by Weidinger et al. in 1984.

Good correlation was found between the observed and expected phenotype distributions, assuming Hardy-Weinberg equilibrium conditions.

No significant differences have been found between the allele frequencies calculated in our population sample and other samples from Continental Italy (Table 2).

Table 3 gives the population data obtained by various authors showing the obvious variation in ethnic and geographic distribution. The estimated frequency of α2HS* 1 allele in our sample and in Continental Italy is higher than the frequency obtained in northern Europe and nothern America but lower than that found in North Africa; it seems closer to the values recorded in Japanese.

On the basis of the allele frequencies estimated in the population sample from Brescia, the cumulative theoretical "a priori" probability of exclusion for α2HS is 15.22% (the I class theoretical "a priori" probability of exclusion is 8.2%).

References

1. Abe S, Hiraiwa K, Sebetan IM (1987) Distribution of α2HS-glycoprotein types in Egyptian. J Anthrop Soc Nippon 95: 137–139
2. Anderson NL, Anderson NG (1977) High resolution two-dimensional electrophoresis of human plasma proteins. Proc Natl Acad Sci USA 74:5421–5425
3. Boutin B, Feng SH, Arnaud P (1985) The genetic polymorphism of $Alpha_2$ HS glycoprotein: study by ultrathin layer isoelectric focusing and immunoblot. Am J Hum Genet 37:1098–1105
4. Cox DW, Andrews BJ (1983) Silver stain immunofixation for α2HS-glycoprotein: a new method for detection of protein heterogeneity. In: Stathokos D (ed) Electrophoresis '82. Walter de Gruyter, Berlin, pp 243–247
5. Cox DW, Andrews BJ, Wills DE (1986) Genetic polymorphism of α2HS glycoprotein. Am J Hum Genet 38:699–706
6. Cox DW, Francke U (1985) Direct assignment of Orosomucoid to human chromosome 9 and α2HS-glycoprotein to chromosome 3 using human fetal liver × rat hepatoma hybrids. Hum Genet 70:109–115
7. Dikson IR, Poole AR, Veis A (1975) Localization of plasma α2HS glycoprotein in mineralising human bone. Nature 256: 430–432
8. Giari A, Demenici R, Bargagna M (1988) Alpha-2-HS glycoprotein polymorphism in Tuscany (Italy). In: Mayr WR (ed) Advances in forensic haemogenetics 2. Springer, Berlin Heidelberg New York, pp 566–569
9. Heremans JF (1960) Les globulines seriques du systeme gamma. Brussels, Arscia: 103–105
10. Kishi K, Yasuda T, Ikehara Y (1988) Alpha-2-HS glycoprotein polymorphism detected in human urine by isoelectric focusing and immunoblotting. Hum Hered 38:207–210
11. Lebreton JP, Joisel F, Raoult JP, Lannuzel B, Rogez JP, Humbert G (1979) Serum concentration of human $Alpha_2$ HS glycoprotein during the inflammatory process. J Clin Invest 64: 1118–1129
12. Lewis JG, André CM (1980) Effect of human α2HS glycoprotein on mouse macrophage function. Immunology 39:317–322
13. Lewis JG, André CM (1981) Enhancement of human monocyte phagocytic function by α2HS glycoprotein. Immunology 42:481–487
14. Lewis JG, André CM (1982) Evidence that the $Alpha_2$-HS glycoprotein binds to lymphocytes transformed by Epstein Barr Virus. FEBS Lett 138:37–39
15. Lewis JG, André CM (1983) Evidence that the α2HS glycoprotein receptor and the Epstein Barr Virus Nuclear Antigen (EBNA) are identical. FEBS Lett 161:306–310
16. Luckenbach C, Kompf J, Ritter H (1988) Polymorphism of AHS-glycoprotein: comparison of different IEF and detection techniques. In: WR Mayr (ed) Advances in forensic haemogenetics 2. Springer, Berlin Heidelberg New York, pp 235–239
17. Olaisen B, Mevag B, Teisberg P, Gedde-Dahl T Jr, Helland P, Siverts A, Jonassen R, Hjalmarsson K, Swan T (1981) Plasma protein polymorphism in two-dimensonal electrofocusing/SDS electrophoresis. 9 Internationale Tagung der Gesellschaft für forensische Blutgruppenkunde e.V. Referate, Bern 29/9 – 3/10. 1981:543
18. Putnam FW (1984) Progress in plasma proteins. In: Putnam FW (ed) The plasma protein, vol 4. Academic Press, New York, pp 1–44
19. Robinet-Levy M, Yuasa I, Umetsu K, Ikebuchi J (1988) Genetic polymorphism of α2HS glycoprotein in a French population. Description of two new rare variants. Hum Hered 38: 74–177
20. Scacchi R, Lucarelli P, Corbo RM (1989) α2HS-glycoprotein phenotype and allele distribution in continental Italy and Sardinia. Hum Hered 39:52–54
21. Schmid K, Burgi W (1961) Preparation and properties of the human plasma Ba_2-α2-glycoproteins. Biochim Biophys Acta 47:440–453
22. Sebetan IM, Heshmat MM (1988) Genetic polymorphism of desialyzed Alpha-2HS glycoprotein by ultrathin isoelectric focusing. Z Rechtsmed 101:205–207
23. Tamaki Y, Fukuda M, Nishimukai H, Kishida T (1985) Application of immunoblotting to serum protein phenotyping with reference to α2HS-glycoprotein (AHS) typing of bloodstains. Z Rechtsmed 95:153–158
24. Tarkkala Mender T, Kühnl P (1986) Alpha-2-HS glycoprotein polymorphisms. In: Brinkmann B, Hennigsen K (eds) Advances in forensic haemogenetics, vol 1. Springer, Berlin Heidelberg New York, pp 167–174
25. Triffit JT, Gebauer V, Ashton B, Owen ME, Reynolds JJ (1976) Origin of $Alpha_2$ HS glycoprotein and its accumulation in bone. Nature 262:226–227
26. Umetsu K, Kashimura S, Ikeda N , Suzuki T (1983) Classification of α2HS glycoprotein (α2HS) types by isoelectric focusing. Z Rechtsmed 91:33–35
27. Umetsu K, Kashimura S, Ikeda N, Suzuki T (1984) A new α2HS – glycoprotein allele (AHS*5) in two Japanese families. Hum Genet 68:264–265
28. Umetsu K, Yusa I, Nishimura H, Sasaki H, Suzuki T (1988) Genetic polymorphisms of orosomucoid and Alpha-2-HS glycoprotein in a Philippine population. Hum Hered 38:287–290
29. Weidinger S, Schwarzfischer F, Burgemeister R, Cleve H (1984) Genetic Alpha-2-HS glycoprotein phenotypes demonstrated by isoelectric focusing and immunofixation. In: Neuhoff V (ed) Electrophoresis '84. Verlag Chemie, Weinheim, pp 487–490
30. Weidinger S (1986) Isoelektrische Fokussierung und Printimmunofixation: Ein schnelles Verfahren zur Typisierung des alpha-2-HS Glykoproteins. In: Elektrophorese Forum '86, Band 6, Discussionstagung, München, 27–29 Oktober 1986
31. Westwood S (1988) The use of α2HS-glycoprotein and group specific component in typing forensic blood samples for discriminative and investigative purposes. Electrophoresis 9: 432–437
32. Yuasa I, Taira T, Suenaga K, Okada K (1985) Determination of α2HS-glycoprotein phenotypes by isoelectric focusing and immunoblotting: polymorphic occurrence of HSGA*5 in Okinawa. Hum Genet 70:32–34
33. Yuasa I, Umetsu K (1988) Genetic polymorphism of α2HS glycoprotein: characterization and application to forensic haemogenetics. Electrophoresis 9:404–410