TECHNICAL NOTE

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Genetic Polymorphism of Alpha-2-HS-Glycoprotein in Northern Bavaria (Germany). Simplified AHSG-Phenotyping by Isoelectric Focusing Using Dry Gels

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ABSTRACT: A simple and practical method of detecting AHSG using isoelectric focusing on dry gels is described. The procedure is both rapid and provides reliable results. AHSG phenotypes were determinated in 215 unrelated individuals from Northern Bavaria. The allele frequencies obtained were: AHSG*1 = 0.7139, AHSG*2 = 0.2697 and AHSG*3 = 0.0164.

KEYWORDS: forensic science, Alpha-2-HS-Glycoprotein, genetic polymorphism, isoelectric focusing, dry gels

The alpha-2-HS glycoprotein (AHSG), an alpha fraction plasma protein, was first described in 1960 [*1*]. Some time later, it was isolated from human plasma. Its chemical composition along with a number of physicochemical properties were analysed by Schmidt et al. [*2*], Heimburger et al. [*3*], and Schultze [*4*]. Despite earlier assumptions, AHSG was found to be comprised of a single polypeptide chain and to have a molecular weight of about 50 kDa. [*5,6*]. AHSG is synthesized in the liver [*7*], and has been found in normal human bone marrow [*8*]. Its average concentration in the plasma is between 40 and 80 mg/dL [*9*]. Changes in the plasma concentration have been found to be associated with certain diseases [*5,8,10*].

A genetic polymorphism of AHSG was first detected in 1977 by means of two-dimensional electrophoresis [*11*]. The electrophoretic types discovered at that time (L and N) correspond to the gene products AHSG 1 and AHSG 2 [*12*]. In 1983, Cox et al. [*13*] demonstrated the genetic polymorphism of AHSG after isoelectric focusing (IEF) and silver-stain immunofixation. In addition to the three alleles AHSG *1, AHSG *2 and AHSG *3, found by these authors, 15 variants have so far been described [*14–19*].

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Owing to the particular distribution of the AHSG alleles in the various populations investigated to date [*18,20–25*], this system is suitable for use as a genetic marker for paternity testing, and for bloodstain analysis. AHSG is a highly stable protein that can be detected even in bloodstains that are many months old [*6*]. According to Kishi [*27*], AHSG can also be detected in human urine.

This paper investigates the suitability of commercially available rehydratable gels (CleanGels, Pharmacia) for the detection of AHSG genotypes. The database of a Northern Bavarian sample is compared with other population studies.

Material and Methods

AHSG phenotype determination in 215 unrelated individuals from Northern Bavaria was carried out by isoelectric focusing using CleanGel IEF (Pharmacia, No. 80-5067-37), a neutral-pH

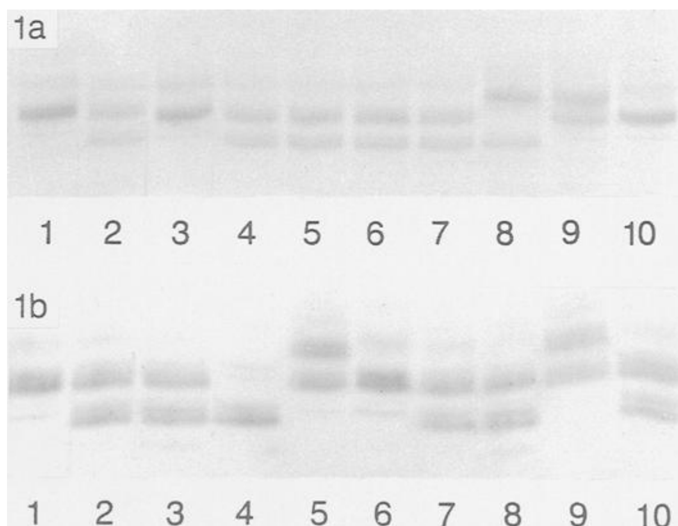

FIG. 1—*AHSG band patterns of native blood samples revealed by IEF on dry gels (CleanGel IEF, Pharmacia).* 1a *(from left to right) 1, 2-1. 1, 2-1, 2-1, 2-1, 2-1, 3-2, 3-1, 1.* 1b *(from left to right) 1, 2-1. 2-1. 2, 3-1, 1, 2-1, 2-1, 3-1, 2-1.*

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dry gel, rehydrated in a solution of carrier ampholyte prior to use. Rehydration was effected over a period of 55 minutes in a solution comprising 2 g sorbitol, 0.1 mL Pharmalyte[R] pH 3–10, 0.2 mL Pharmalyte[R] pH 5–6, 0.7 mL Pharmalyte[R] pH 4.5–5.4, and 17 mL distilled water. A 10 μL serum sample was incubated with 10 μL neuraminidase (Boehringer, No. 107590, 1 mg and 1 mL bidest.) overnight at 4°C.

TABLE 1—*AHSG phenotype distribution and allele frequencies in Northern Bavaria.*

Phenotype	Observed *n*	Expected *n*	Allele frequencies
1	104	109.57	AHSG*1 = 0.7139
2-1	93	82.79	AHSG*2 = 0.2697
2	11	15.63	AHSG*3 = 0.0164
3-1	6	5.03	
3-2	1	0.008	
Total	215	213.028	

$\chi^2 = 3.1008$, 2 d.f.: $0.2 < P < 03$.

For the IEF, 5 μL samples were placed on applicator strips at a distance of approximately 1.5 cm from the cathode, and separated under the following conditions:

a) 500 V / 8 mA / 8 W / 10°C / 45 min
b) 2350 V / 14 mA / 14 W / 10°C / 105 min
c) 2550 V / 14 mA / 14 W / 10°C / 45 min

Diffusion blotting was carried out on an NC membrane (cellulose nitrate (E), Schleicher and Schuell, No. 401199, BA 85, 0.45 μm) for 35 min, followed by blocking with 0.1% bactogelatine (400 mg Bactogelatine (Difco) ad 400 mL TBS Tween Buffer) for 30 min.

Immunofixation was performed using an anti-human alpha-2-HS-glycoprotein (Boehringer, OTE 04/05 from the rabbit) for overnight incubation (dilution: 1 to 1,000 in TBS Tween pH 10.3). This was followed by three five-minute rinses with TBS Tween buffer (pH 10.3). After which, incubation was effected with peroxidase-conjugated anti-rabbit immunoglobulins (from pig, DAKO, Code P217, dilution 1 to 1,000 in TBS Tween (pH 10.3) for three hours (shaking). Following this, the samples underwent three rinses

TABLE 2—*Distribution of AHSG allele frequencies in various populations.*

Populations	*n*	Allele frequencies			References
		AHSG*1	AHSG*2	Others	
Afro-Carib.	71	0.6901	0.2606		(16)
Afro-Carib.	119	0.6597	0.235	AHSG*10:0.0966 others: 0.0084	(32)
American	150	0.6533	0.3467		(34)
Canadian	215	0.6419	0.3535	AHSG*3: 0.0046	(16)
Chinese	286	0.6958	0.3042		(31)
English	382	0.6466	0.3469	AHSG*3: 0.0052 AHSG*10: 0.0013	(32)
French	240	0.7167	0.2750	0.0083	(18)
German					
N.-Bavaria	215	0.7139	0.2689	AHSG*3 0.0163	this study
Hessen	197	0.655	0.340	0.005	(33)
South-G.	344	0.6642	0.3208	AHSG*3: 0.0072 AHSG*4: 0.0058 AHSG*10: 0.0020	(17)
South-G.	368	0.6889	0.3016	0.0095	(25)
South-W.-G.	249	0.631	0.363	0.006	(35)
Münster	168	0.6250	0.3750		(24)
Indian	205	0.8073	0.18781	AHSG*3: 0.0049	(32)
Italian					
Lombardy	700	0.7472	0.2507	0.0021	(20)
Rome	199	0.756	0.244		(21)
L'Aquila	106	0.788	0.212		(21)
Sardinia	152	0.832	0.168		(21)
Japanese					
Northern-J.	1.003	0.7338	0.2662		(15)
Fukuoka	1.074	0.7165	0.2817	0.0019	(19)
Tsushima	514	0.6916	0.3045	0.0039	(19)
Koshiki	103	0.7282	0.2718		(19)
Ishigaki Is	332	0.7922	0.18671	AHSG*5: 0.0151 others: 0.0060	(24)
Fukui	256	0.7637	0.2363		(27)
Okihawa	397	0.7670	0.2065	AHSG*5: 0.0264	(36)
Yamaguchi	400	0.7325	0.2675		(36)
Izumo	300	0.7233	0.2767		(36)
Oita	283	0.7456	0.2544		(26)
Libyans	110	0.8364	0.1636		(22)
Spanish					
Galicia	506	0.7559	0.2441		(30)
Taiwanese	199	0.7286	0.2714		(24)
Philipino	115	0.6870	0.3130		(23)

in TBS Tween (pH 10.3) and a brief rinse in sodium acetate buffer (pH 5). The development solution comprises 20 mg 3-amino-9-ethylcarbazole, 2.5 mL acetone, 50 μL H_2O_2, and 50 mL sodium acetate buffer (pH 5). The development time was 10 min (stopped with tap water).

Results and Discussion

Typical results obtained with fresh blood samples are shown in Fig. 1. In Table 1 the frequencies of the phenotypes in the population of Northern Bavaria (n = 215) is shown. According to our findings, the allele 3 is conspicuously more frequent compared with other German population studies (Table 2), whereas the AHSG*2 value obtained was relatively low. Rare variants have not been observed so far in our study. The differences between the German populations for alleles 2 and 3 were statistically significant ($P = 0.027$, $\chi^2 = 23.06$, d.f = 12). Pairwise comparisons were significant for Münster/N-Bavaria ($P = 0.002$), Münster/South-Germany ($P = 0.04$) Münster/ South-West-Germany ($P = 0.008$) and N-Bavaria/Hessen ($P = 0.04$). After Bonferroni Correction [37] ($P_{bonf} = 10*p$) the difference between Münster (North-Germany) and N-Bavaria (South-Germany) remains significant.

The method described is simple as well as reliable, and necessitates no polymerization of acrylamides, hence toxic exposure to laboratory assistants can be avoided. The method is especially useful in detection of the gene products of the allele AHSG*3 as shown in Fig. 1. This may explain the fact, that in our findings, the phenotype 3-1 and 3-2 were more frequently detected than in other studies.

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