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# Formal and population genetic studies of AHSG: further data from Galicia

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**Summary.** A sensitive immunodetection method for Alpha-2-HS glycoprotein (AHSG) after ultrathin layer polyacrylamide gel isoelectric focusing has been applied to a family study of 126 matings including 292 offspring. Formal genetic studies are in agreement with an autosomal mode of inheritance for this system. A population study of 506 unrelated individuals from Galicia (NW Spain) gave the following frequencies: AHSG*1 = 0.7559 and AHSG*2 = 0.2441, which correspond to a exclusion chance for non-fathers of 0.1505.

**Key words:** Genetic transmission – Genetic marker – Alpha-2-HS glycoprotein – AHSG – Isoelectric Focusing

**Zusammenfassung.** Eine empfindliche immunologische Nachweismethode für die Alpha-2-HS-Glykoprotein (AHSG)-Diagnose nach Ultradünnschicht-Polyacrylamid-Gel-Isoelektrofokussierung wurde in einer Familienstudie angewandt, welche 126 Elternverbindungen und 292 Kinder umfaßte. Die formalgenetischen Untersuchungen stehen mit der Annahme eines autosomalen Erbgangs in Übereinstimmung. Eine populationsgenetische Untersuchung von 506 unverwandten Personen von Galicia (Nordwest-Spanien) ergab die folgenden Genfrequenzen: AHSG*1 = 0,7559 und AHSG*2 = 0,2441; diese Frequenzen entsprechen einer Ausschließungschance für Nicht-Väter von 0,1505.

**Schlüsselwörter:** Genetische Transmission – Genetischer Marker – Alpha-2-HS-Glykoprotein – AHSG – Isoelektrische Fokussierung

## Introduction

The original method of Anderson and Anderson (1977) for the detection of genetic variants of AHSG could be considerably improved using isoelectric focusing and subsequent immunofixation or immunoblotting (Cox and Andrews 1983; Umetsu et al. 1983). At least 22 allelic variants have been described (Fukuma et al. 1991) and the different world populations so far analysed show a relatively high degree of polymorphism (h) ranging from 0.48 for Norwegians (Olaisen et al. 1981) to 0.24 for Egyptians (Abe et al. 1987). The aim of this study was to provide further data of population genetics and family studies to serve as background for forensic and other analyses.

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## Materials and methods

Blood samples were collected from 126 families and 506 unrelated healthy individuals from the Galician population (NW Spain). AHSG phenotype determination was made by isoelectric focusing in 0.4 mm polyacrylamide gels (T = 5, C = 3, sucrose 12% w/v) using ampholytes pH 4–6 (5%, v/v). Gels were prefocused at 12 W constant power, 2,000 V and unlimited current for 30 min. Diluted native plasma samples (1:1) were applied 1 cm from the cathode using Whatmann No. 1 filter paper. IEF was continued for a further 150 min.

For AHSG immunofixation, 2 µl/cm$^2$ of antiserum (anti-AHSG, Atlantic Antibodies, Scarborough, USA) diluted in physiological saline (1/5, v/v) was applied on the gel surface 2–5 cm from the anode. The gel was incubated in a moist chamber at 37°C for 60 min and washed overnight in physiological saline. Visualization of immunoprecipitates was made by means of a simplified silver staining (Budowle and Scott 1985) with the following minor modification: after the potassium dichromate step washing was carried out for 7 min using milli-Q water. Treatment with silver nitrate was increased to 0.2% (w/v) for 10 min, and formaldehyde concentration was 1.66% (w/v) in the reduction step.

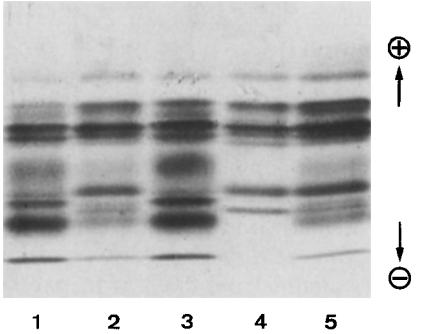


**Fig. 1.** IEF and silver stain immunofixation of AHSG phenotypes. AHSG 1(4); AHSG 2–1(2, 5); AHSG 2(1, 3)

**Table 1.** Distribution of AHSG phenotypes in 126 family groups from Galicia. Expected frequencies are put in parenthesis

| Matings | | | | Offspring | | | | $\chi^2$ | $P$ | d.f. |
|---|---|---|---|---|---|---|---|---|---|---|
| Father | | Mother | N | N | 1 | 2–1 | 2 | | | |
| 1 | × | 1 | 40 | 85 | 85 | – | – | | | |
| 2–1 | × | 1 | 30 | 71 | 32 (35.5) | 39 (35.5) | – | 0.690 | $0.4 < P < 0.5$ | 1 |
| 1 | × | 2–1 | 26 | 56 | 29 (28.0) | 27 (28.0) | – | 0.072 | $0.7 < P < 0.8$ | 1 |
| 2–1 | × | 2–1 | 16 | 42 | 9 (10.5) | 20 (21.0) | 13 (10.5) | 0.857 | $0.6 < P < 0.7$ | 2 |
| 2–1 | × | 2 | 3 | 14 | – | 8 (7.0) | 6 (7.0) | 0.286 | $0.5 < P < 0.6$ | 1 |
| 2 | × | 2–1 | 5 | 11 | – | 4 (5.5) | 7 (5.5) | 0.818 | $0.3 < P < 0.4$ | 1 |
| 2 | × | 1 | 4 | 8 | – | 8 | – | | | |
| 1 | × | 2 | 2 | 5 | – | 5 | – | | | |
| Total | | | 126 | 292 | 155 | 111 | 26 | | | |

**Table 2.** AHSG phenotypes and gene frequencies in Galicia. Expected values are put in parenthesis

| Phenotypes | Phenotype frequencies | Gene frequencies |
|---|---|---|
| AHSG 1 | 286 (289.13) | AHSG*1: 0.7559 |
| AHSG 2–1 | 193 (186.73) | AHSG*2: 0.2441 |
| AHSG 2 | 27 (30.14) | |
| Total | 506 (506.00) | |
| | $\chi^2 = 0.5721$, $0.3 < P < 0.5$, 1 d.f. | |

## Results and discussion

The results shown in Table 1 indicate that there are 2 autosomal codominant alleles (AHSG*1 and AHSG*2). Our findings are in agreement with previous family studies (Umetsu et al. 1983; Weidinger et al. 1984; Boutin et al. 1985; Luckenbach et al. 1988; Cox et al. 1986) since there are no exceptions to this hypothesis from all the offspring examined and the segregation ratios are concordant with Mendelian expectations. In the particular case of matings AHSG 1 × 2–1 an excess of heterozygotes (and consequently a deficit of homozygotes AHSG 1) was observed in this study ($\chi^2 = 0.1969$, $0.2 < P < 0.3$, 1 d.f.). Analogous results were registered in other family studies (Cox et al. 1986; Luckenbach et al. 1988). The pooled data from these three studies gives a value of $\chi^2 = 3.729$ ($0.05 < P < 0.1$, 1 d.f.) which, even though this does not classify as a significant statistical difference, is very close to the significance level.

The phenotype frequency distribution of AHSG in 506 unrelated individuals from the Galician population is summarized in Table 2. A good fit between empirical an theoretical phenotype frequencies according to the Hardy-Weinberg law was observed ($\chi^2 = 0.5721$, $0.3 < P < 0.5$, 1 d.f.). The heterogeneity of gene frequencies among European populations could lead to significant differences in some cases. For this reason the extrapolation in the allelic frequencies from nearby populations, as a basis for estimating the statistical parameters in paternity testing and in the analysis of bloodstains, should be carried out with a considerable amount of precaution. In our study, the AHSG locus defines a chance of exclusion of paternity Ce = 0.1505. This value is higher than other routine genetic markers such as BF, PLG, C3 or subtyped ESD, which reveals the value of this system for forensic studies.

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