

α_2 -HS-GLYCOPROTEIN (A₂HS): IMPROVED PHENOTYPING BY FOCUSING IN AN
IMMOBILIZED PH-GRADIENT. ALLELE FREQUENCIES IN A SOUTH GERMAN POPULATION
SAMPLE AND LINKAGE DATA FROM A LARGE PEDIGREE

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INTRODUCTION

The use of A₂HS as an additional polymorphic marker in paternity testing and related forensic applications is not yet introduced in the routine expertises of all laboratories. One of several causes is the problem of antiserum supply, another the poor and batch-dependent resolution of the specific bands by separation with commercial carrier ampholytes according to previously described procedures (Weidinger, 1986; Mendner and Kühnl, 1986). Thus we developed an appropriate immobilized pH-gradient, which allows an improved and better reproducible separation.

METHODS

Separation data

Gel dimensions: 150 x 120 x 0.5 mm

Casting chamber: Silicon rubber U-frame, Mylar and Gel-Fix sheets (LKB, SERVA) between glass plates.

Gradient mixer: 2 x 7 ml, from Hölzel, Dorfen, FRG

Gel composition: Acidic dense solution: 290 μ l Immobiline pK 4.6, 100 μ l pK 9.3, 1600 μ l 87% glycerol, 1500 μ l water; basic light solution: 250 μ l pK 4.6, 230 μ l pK 6.2, 3100 μ l water; either solution 1250 μ l acrylamide 20 T 3 C. pH was 4.2 and 5.15 before addition of catalysts (3.75 μ l TEMED and 10 μ l 10% persulfate to 4500 μ l in each chamber.

Casting and gel processing: Gels were cast without pumping according to procedures suggested by LKB (Görg, 1983), left in place to polymerize for 15 min. at room temperature, then polymerization was completed at -50°C for 1 hour. 3 washes with water, 1 with 2% glycerol, each about 10 min, removed unpolymerized material. After complete drying at 50°C, the gels were stored frozen until use. Rehydration was allowed for at least 1 hour with 20% glycerol containing 2 mM Tris-base in the casting chamber.

Samples: Serum or EDTA-plasma, 4 μ l applied on 4x7 mm filter papers 1-1.5 cm beside the cathodal strip

Running conditions: Anolyt 10 mM glutamic acid, catholyt 10 mM NaOH; coolant held at 4 °C; 3000 V, 2 mA, 5 W (maximum settings) over night (16-18 hrs.) no prerun.

Print immunofixation

Material: The antiserum is not commercially available (in Germany), but on request from the research department of Behring(Höchst) / Calbiochem via their regional distributors. The CAF membrane was from Sartorius, type 11200, 57 x 145 or 80 x 150 mm.

Immunofixation: The membrane is soaked with saline, excess moisture removed and the membrane carefully (with no air bubbles trapped) laid on the separation gel, from 1 cm beside the application area towards the anode. 4 µl/cm² of the undiluted antiserum are immediately distributed on the wet membrane with a Drygalski spatula. After 20 min incubation in the moist chamber at 37°C the membrane is removed, washed 3 x 10 min. in saline and stained with coomassie blue as usual. Destaining was done under control before the membrane was transferred to the storage bath (10% acetic acid with 2% glycerol), which was found to result in a more evenly stained membrane.

RESULTS AND DISCUSSION

Separation

As shown in figs. 1A and 1B, the immobilized gradient enlarges the A2HS-region to a distance of about 5 cm, including the anodal band of A2HS 4 and the most cathodal band of A2HS 2. The resolution allows to confirm a certain phenotype on at least three iso-pH levels in a reliable and ampholyte-batch independent way. Simultaneous typing of GC and PI is, in our experience, better to be performed on a separate (part of the) gel to avoid difficulties due to the antiserum background.

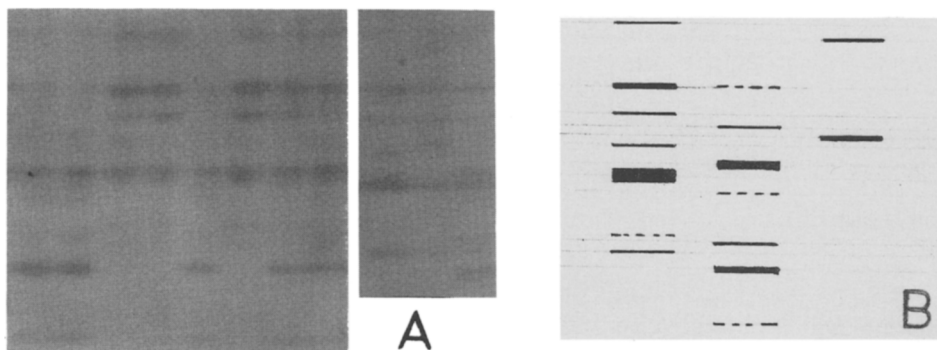


Fig. 1. A: Photographic representation of A2HS phenotypes. Lanes 1,2,5: A2HS 2; lanes 3,4,6,9: A2HS 1; lanes 7,8,11: A2HS 2-1; lane 10: A2HS 4-1. B: Schematic presentation of the bands characteristic for alleles 1,2, and 4 (from left to right, the anode is at top)

Segregation and linkage data

Though Eiberg (1983) reported close linkage of TF and A2HS, the analysis of a large pedigree (kindly performed by Dr. T.F. Wienker, University of Freiburg, FRG) does not show convincing values in favor of close linkage, $\zeta=0.093$ at $\theta=0.23$. One of the three informative pedigrees (figs. 2 A,B,C) shows an crossover (no sign of illegitimacy in 27 systems including HLA - candidates for a intrafamilial bone marrow transplantation). Neither the segregation data of the pedigrees nor the other families investigated revealed a deviation from the assumed autosomal codominant inheritance nor did we observe incompatible mother-child pairs or single contrary homozygosity exclusions in about 100 cases of disputed paternity.

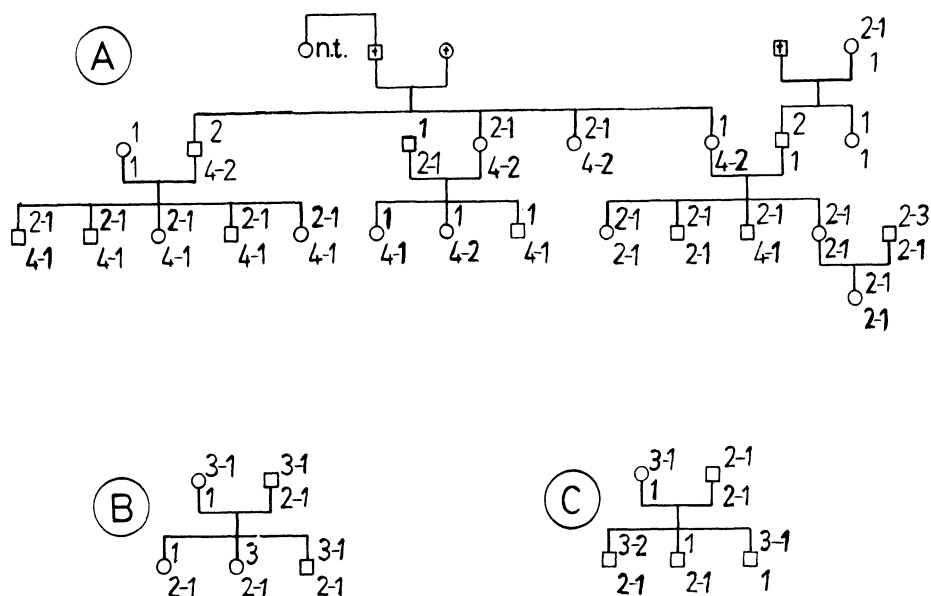


Fig. 2. A. 4-generations pedigree from a family originating from the surroundings of Stuttgart. Two persons in generation 1 come from the same little village, thus it can be assumed, that they both carried the A2HS*4. B. In this family a crossover has to be assumed. Numbers above the person's symbols refer to the TF-C subtype, those below to A2HS. C. Same as in B.

Phenotype and allele frequencies

Data from the south german population sample from the region of Ulm (Oberschwaben, Schwaben, Ostwürttemberg) are shown in table 1, they fit well with other population samples from Germany and other caucasian populations, 0.64-0.66 for A2HS*1 and 0.32-0.34 for A2HS*2. Three rare variants could be observed, most frequently A2HS*4. Data from an isolate population from our region ("Steinheimer Wald") are shown in table 2. Though the isolate is

very small and therefore the number of persons limited (20 out of 5 families), there is a significant difference to other german population samples. This was not observed for the other systems tested except HP.

Table 1. A2HS phenotype and allele frequencies in the region of Ulm (Ostwürttemberg, Schwaben, Oberschwaben), 368 unrelated germans.

phenotype	n	obs.	%	n	exp.	%
1	179		48.64	174.6		47.46
2-1	144		39.13	152.9		41.56
2	38		10.33	33.5		9.11
1-Var	5		1.36	4.8		1.30
2-Var	2		0.54	2.1		0.57
Var	0		0	0.03		0.01

A2HS* 1 : 0.6889
 A2HS* 2 : 0.3016
 A2HS* Var 0.0095
 $\Sigma \chi^2 = 1.29$

Table 2. A2HS phenotype frequencies in an isolate population sample from the region of Ulm ("Steinheimer Wald")

phenotype	n	obs.	%	% exp.(from table 1)
1	16		80.0	47.5
2-1	4		20.0	41.6
others	0		0	10.9

SUMMARY

A2HS proved to be of great value as additional system in parentage testing (with the exclusion chance of 17.8% according to Weidinger (1986)) as well as for other problems. With the reliable and reproducible separation method given here, the system should now be generally included in the standard expertises as it was already suggested.

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