The Distribution of Hp Subtypes in Sweden

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INTRODUCTION

Smithies et al. (1962) showed the existence of haptoglobin polymorphism. Population studies of the polymorphism have in general been restricted to the identification of the three phenotypes Hp1, Hp2-1 and Hp2. Technical improvements have made it possible to detect Hp subtypes.

The haptoglobin molecule consists of α- and β-chains linked with disulfide bridges. The isoelectric heterogeneity of the β-chain is due to its content of sialic acid. The α-chain is polymorphic, and the most common subalteles 1S, 1F, 2FS, 2SS and 2FF may be separated by isoelectric focusing.

In 1985 Teige et al. presented a method for Hp subtyping, which is very well adapted for routine investigations of cases of disputed paternity.

The aim of this study is to present the distribution of haptoglobin subtypes and allele frequencies from unrelated adult Swedes. A family and a mother-child material are also presented.

MATERIAL and METHODS

Serum samples from 564 unrelated Swedish adults and a mother-child material consisting of 113 pairs were investigated. All persons were involved in paternity cases. Sera from 27 families with 62 children were also examined. The family material originated from northern Sweden and was involved in a diabetes mellitus (IDDM) investigation, including IDDM families as well as control families.

After arrival at the laboratory the serum was separated from the blood cells by centrifugation and stored at +4°C. The subtypes were determined twice or more.

The determination of Hp subtypes was carried out by isoelectric focusing followed by enzyme linked immunoblotting technique mainly according to Teige et al (1985).

Advances in Forensic Haemogenetics 2
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Sample Treatment

Serum samples were reduced and neuraminidase treated according to Teige et al. (1985).

Gel Preparation

110 x 240 x 0.5 mm polyacrylamide gels (T=4 %, C=3 %) were cast on Gel Bond (FMC Corporation, USA) film and polymerized with TEMED and ammoniumpersulphate. The gels contained 3.4 % of an Ampholine (LKB, Bromma, Sweden) mixture of 3 parts pH 3.5-10, 2 parts pH 4-6 and 1.5 parts pH 6-8.

Isoelectric Focusing

The anode solution was 1 M H_3PO_4 and the cathode solution was 1 M NaOH. The cooling temperature was +10°C. The gels were pre-focused for 1 h with maximum settings at 500 V and 8 W before the samples were applied (Wh1 7x4 mm) 0.5 cm from the cathode. The filter papers were removed after 45 minutes focusing with maximum settings at 1300 V and 10 W. Then the isofocusing was continued for 1.5 h with maximum settings at 1800 V and 16 W.

Blotting Procedure

The proteins were transferred to nitrocellulose sheets (Bio-Rad, Richmond, USA) by passive blot for 1 h at room temperature. Thus the nitrocellulose paper was presoaked in 40 mM Tris, 192 mM glycine, 0.01 % SDS buffer, pH 8.7 before placing on top of the gel, followed by layers of filter papers wetted in the blotting buffer, and some layers of dry filter papers.

Visualization

The nitrocellulose paper was washed in 0.15 % Tween 20, 48 mM Na_2HPO_4, 18 mM KH_2PO_4, 147 mM NaCl buffer, pH 7.2 (PBS-Tween buffer) for 2 h. The paper was shaken over night in a solution of peroxidase conjugated goat-anti-human Hp (Cappel, Worthington) diluted 6/1000 with PBS-Tween buffer and washed for 1 h in PBS-Tween buffer. The development was performed in 50 ml of a 0.2 M NaCl, 59 mM Tris-HCl buffer, ph 7.4, 30 mg 4-chloro-1-naphtol, 2 ml acetone and 30 µl 20 % H_2O_2. The colour reaction was stopped with water.

In contrast to the method by Teige et al. (1985) one antibody is used instead of the two-step antibody technique whereby the method will be less time-consuming and laborious.

RESULTS and DISCUSSION

The phenotype pattern is shown in Fig. 1. The distribution of Hp phenotypes and gene frequencies in the population in Sweden are presented in Table 1. The population was in Hardy-Weinberg equilibrium with a chi-square = 6.5 $0.1<p<0.2$ at 4 d.f.

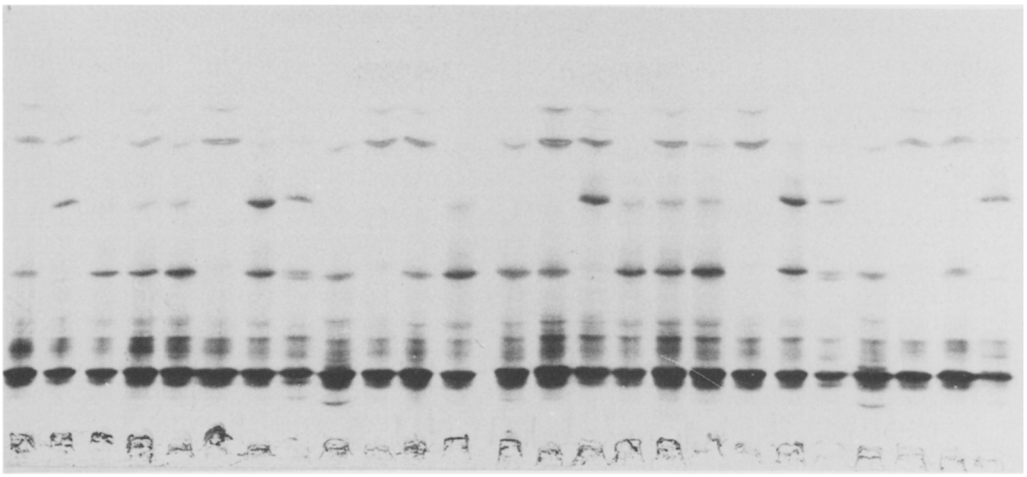

Fig. 1. Haptoglobin phenotypes in neuraminidase treated serum samples revealed by isoelectric focusing and immunoblotting procedures. Hp phenotypes from left to right: 1F-1S, 2SS-1S, 2FS, 2FS-1S, 2FS-1F, 1S, 2FS-2SS, 2SS-1F, 2FF-1F, 1S, 1F-1S, 2FS, 1F, 1F-1S, 2SS-1S, 2FS, 2FS-1S, 2FS-1F, 1S, 2FS-2SS, 2SS-1F, 2FF-1F, 1S, 1F-1S and 2SS.

The Hp allele frequencies agree well with the data published from other populations in Europe (Thymann 1977; Shibata et al. 1982; Teige et al. 1985). Hp subtyping of 113 mother-child pairs has been investigated. Unexpected combinations in the material are not found. The data are in full accordance with the postulated genetic model of an autosomal locus with codominant alleles.

The results of the examination of 27 families with 62 children are given in Table 2. In each combination group of parental types, the segregation of phenotypes in the children is in accordance with the assumption of autosomal, codominant inheritance.

The isofocusing/immunoblotting method suits a routine laboratory very well because no purification of the haptoglobin molecule prior to isofocusing and only one dimensional electrophoresis are required. The determination of Hp subtypes extends the number of common alleles from two to five and the theoretical chance of exclusion of non-fathers from approximately 18 % to 30 %.

The Hp system is now very informative and classification results are highly reproducible. The system is useful for both population studies and cases of disputed paternity.

Table 1. Distribution of Hp phenotypes in Sweden

Hp-types	Observed n	Expected n	Gene frequency
1S	37	30.20	Hp^{1S} = 0.231
1F	9	13.73	
1F-1S	44	40.72	Hp^{1F} = 0.156
2FS	192	183.82	
2FS-2SS	25	26.27	Hp^{2FS} = 0.571
2FS-2FF	0*	0.58	
2SS-2FF	0*	0.05	Hp^{2SS} = 0.041
2SS	0*	0.94	
2FF	0*	0.00	Hp^{2FF} = 0.001
2FS-1S	131	149.02	
2FS-1F	104	100.46	
2SS-1S	11	10.65	
2SS-1F	10*	7.18	
2FF-1S	1*	0.23	
2FF-1F	0*	0.16	
Total	564	564.01	

χ^2 = 6.5 $0.1<p<0.2$ 4 d.f.

* Subtypes are pooled in one group according to the table for the χ^2-analysis.

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Table 2. Segregation of Hp subtypes in 27 families with 62 children

Matings		n	Children (n=62) 1F	1S-1F	2FS	2FS-1S	2FS-1F	2FS-2SS	2SS-1S
1F	x 1F	1	3	-	-	-	-	-	-
2FS	x 2FS	4	-	-	13	-	-	-	-
2FS	x 2FS-1S	3	-	-	3	2	-	-	-
2FS	x 2FS-1F	5	-	-	8	-	3	-	-
2FS	x 2SS-1F	2	-	-	-	-	3	0	-
2FS	x 1F	1	-	-	-	-	2	-	-
2FS	x 1S	2	-	-	-	4	-	-	-
2FS-1S	x 2FS-1F	3	-	3	1	3	1	-	-
2FS-1S	x 2SS	1	-	-	-	-	-	1	1
2FS-1S	x 2FS-2SS	1	-	-	0	5	-	1	0
2FS-1F	x 2FS-1F	1	1	-	0	-	0	-	-
2FS-1F	x 1S-1F	1	0	-	-	1	0	-	-
2FS-1F	x 1S	1	-	2	-	0	-	-	-
2FS-2SS	x 2FS-1F	1	-	-	0	-	1	0	-
Total		27	4	5	25	15	10	2	1