Haptoglobin Subtypes in Lower Saxony (Germany)

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INTRODUCTION

The two-allelic polymorphism of the haptoglobin system has been used in cases of disputed paternity for more than thirty years. A lack of methods suitable for routine subtyping of the Hp α-chains hindered it from being commonly used in paternity testing. Today various methods, based on somewhat different principles, have been established. Patzelt and Schröder (1985), inspired by the work of Shibata (1982), developed a time-saving procedure of Hp subtyping by isoelectric focussing of haptoglobin cleavage products. We are performing this technique with slight modifications in routine paternity testing. The aim of this study is to present the distribution of the Hp polymorphism in 431 unrelated adults from Lower Saxony.

MATERIALS AND METHODS

Sera:
Sera were obtained from probands involved in paternity testing.

Hp purification:
60 µl (in some cases 100 µl) of non-hemolytic serum were mixed with 2 ml DEAE SS-cellulose suspension (sodium acetate buffer 0.01 M, pH 4.7) and incubated for at least 40 min. After short centrifugation, the pellet was washed twice with sodium acetate buffer. The pellet was resuspended with 50 µl of 0.125 M acetic ammonium solution and centrifuged again to elute the hemoglobin molecules.

Reductive cleavage:
40 µl of the haptoglobin-containing supernatant were mixed with 20 µl of the reductive reagent (8 M urea in 0.1 M boric acid, 0.04 M sodium hydroxide, pH 8.8, 1.5 M dithiothreit), incubated for at least 30 min at 37°C, and alkylated with 8 µl 0.5 M iodoacetamide solution.

Isoelectric focussing and staining:
IEF was performed in polyacrylamide gels (T=5.5%, C=3%; 260x125x0.5 mm) containing carrier ampholytes (Ampholine LKB; 0.6 ml of pH 5-7, 0.2 ml of pH 6-8, 0.2 ml of pH 3.5-5, and 0.1 ml of pH 3-10 in 16 ml gel volume). IEF was performed for 105 min at a maximum power supply of 1600 V, 10 mA, 15 W without prefocussing. Fixation, staining and destaining of the gels were performed according to Steck et al. (1980).

RESULTS

The Hp phenotype distribution among 431 unrelated individuals and the corresponding gene frequencies were investigated (Tab. 1). The Hp phenotype distribution as well as the allele frequencies obtained are in agreement with those expected according to the Hardy-Weinberg law. Furthermore, the Hp phenotypes in 171 mother-child pairs were analysed. No mother-child exclusion was observed. The calculated Hp allele frequencies were compared to published data of different studies (Tab. 3). The evaluated data of the allele frequencies correspond to those obtained by investigators for other parts of Germany. However, in the investigated popu-

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lation of Lower Saxony, Hp 1F and Hp 2SS show slightly higher values, whereas Hp 1S and Hp 2FS are slightly decreased as compared to the corresponding values in the other regions of Germany.

Tab. 1: Hp phenotype distribution

Hp subtype	Observed n	Observed %	Expected	Allelic frequencies
1F	6	1.392	10.102	
1F-1S	36	2.353	31.867	
1S	22	5.104	24.846	Hp *1F = 0.153
1F-2FF	-	-	0.306	*1S = 0.240
1F-2FS	79	18.329	75.013	*2FS = 0.568
1F-2SS	5	1.160	4.751	*2SS = 0.036
1S-2FF	-	-	0.482	*2FF = 0.002
1S-2FS	117	27.146	117.640	
1S-2SS	10	2.320	7.451	Σ = 0.999
2FF	-	-	0.002	
2FF-2FS	2	0.464	1.137	Chi^2 = 5.877
2FF-2SS	-	-	0.072	df = 10
2FS	138	32.018	139.678	p = 0.825
2FS-2SS	16	3.712	17.639	
2SS	-	-	0.559	
	431	99.999	431.545	

Tab. 2: Hp phenotypes in 171 mother-child pairs

child	1F	1F-1S	1S	1F-2FS	1F-2SS	1S-2FS	1S-2SS	2FS	2FS-2SS
mother									
1F	-	1	-	-	-	-	-	-	-
1F-1S	-	4	1	7	-	2	-	-	-
1S	-	-	3	-	-	7	-	-	-
1F-2FF	-	-	-	-	-	-	-	-	-
1F-2FS	3	3	-	11	-	3	-	10	1
1F-2SS	-	-	-	-	-	-	-	-	-
1S-2FF	-	-	-	-	-	-	-	-	-
1S-2FS	-	7	6	4	-	17	-	12	2
1S-2SS	-	-	2	-	-	3	1	-	-
2F-2FS	-	-	-	1	-	-	-	-	-
2FF-2SS	-	-	-	-	-	-	-	-	-
2FS	-	-	-	13	-	7	-	34	-
2FS-2SS	-	-	-	-	2	-	1	1	2
2SS	-	-	-	-	-	-	-	-	-
Total	3	15	12	36	2	39	2	57	5

Tab. 3: Hp allele frequencies of different studies

	n	1F	1S	2FS	2SS	2FF
Patzelt and Schröder (1985) Berlin	1275	0.1471	0.2502	0.5753	0.0251	0.0020
Bertrams et al. (1987) Rhine-Ruhr	1035	0.1387	0.2538	0.5864	0.0196	0.0015
Zischler et al. (1987) South-Germany	182	0.144	0.254	0.574	0.024	0.0004
Rothämel et al. (1989) Lower Saxony	1500	0.1537	0.2523	0.5620	0.0290	0.002
This study	431	0.153	0.240	0.568	0.036	0.002

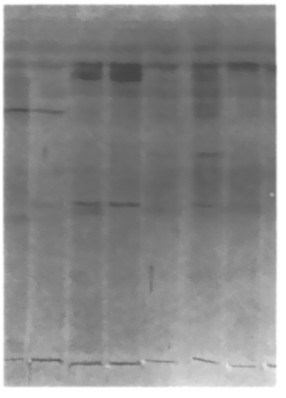

Fig. 1: Haptoglobin subtypes from left to right:
1S, 1S-2FS, 1F-2FS, 2FS, 1F-2SS, 2FS-2SS, 1F-2FS

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