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Patterns and frequencies of common and rare Hp α-chain variants

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INTRODUCTION

The α-chain polymorphism of the serum protein haptoglobin (Hp) has for a long time been used in cases of disputed paternity. The most common Hp α-chain alleles are Hp1 and Hp2, which by further subtyping can be divided into Hp1S, Hp1F, Hp2FS, Hp2SS and Hp2FF.

In cases of disputed paternity conventional Hp typing offers an exclusion efficiency that is about half of the efficiency expected with Hp subtyping. But until a few years ago all available Hp subtyping methods required a purification step before the electrophoretic separation and identification could be done, and in most laboratories routine subtyping of haptoglobin was not performed.

An isofocusing/immunoblot method, requiring no prior purification of the Hp molecule (Teige 1985), has from 1985 been used routinely at the Institute of Forensic Medicine, Oslo, in all cases of disputed paternity as well as in some cases of criminal investigation. During this period one new β-chain variant and three new α-chain variants have been detected. The β-chain variant has already been described (Teige 1986), and the α-chain variants will be shown here. We will further give the Hp phenotype distribution and the allele frequencies obtained by subtyping 3318 unrelated individuals. The exclusion efficiency of the haptoglobin subtyping system is also evaluated.

METHOD

The method has previously been described in detail (Teige 1985). The following antibodies are now used:
Anti-human haptoglobin produced in rabbit, from Dakopatts A/S, P.B. 1359, 2600 Glostrup, Denmark.
Anti-rabbit IgG conjugated to alkaline phosphatase, produced in swine, from Orion Diagnostica, Espoo, Finland.
The alkaline phosphatase staining is performed according to Blake (1984).

Molecular weights and isoelectric points of variants are determined with two-dimensional SDS electrophoresis as described by Olaisen (1981).

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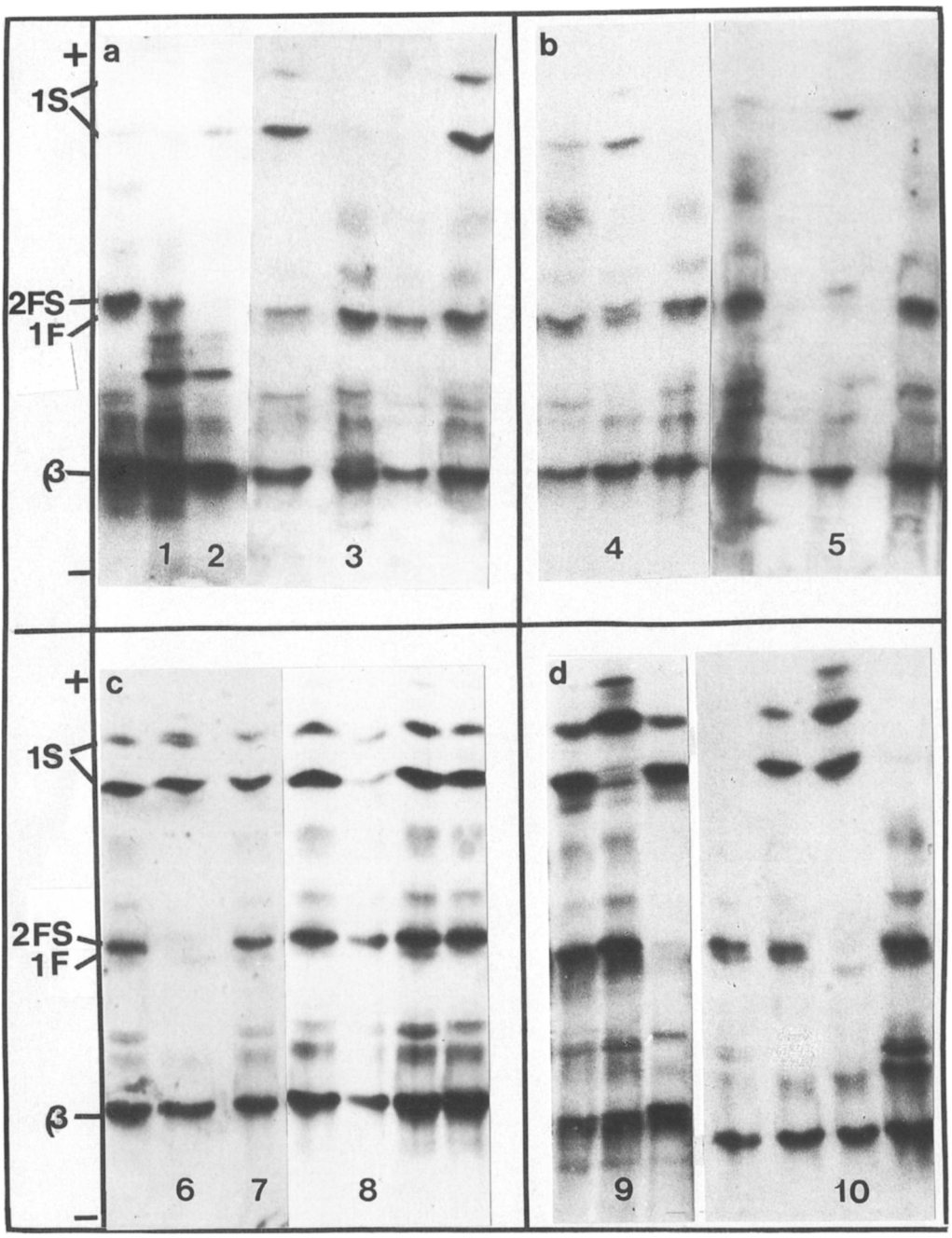

Figure 1. Isofocusing patterns of common and rare haptoglobin α-chain variants. Lane 1: 31F(mother) Lane 2: 31S(child) Lane 3: 2FS2FF Lane 4: Mixture of normal 2FS1S and 2var1S Lane 5: 2var1S Lane 6: 2FS1Svar(alleged father) Lane 7: 1S1Svar(child) Lane 8: 2F1S(mother) Lane 9: 2FS1var(child) Lane 10: 1S1var(mother's husband)

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RESULTS

In Fig. 1 is shown the isofocusing pattern of rare and common Hp -chain variants. The method offers easily recognizable patterns of each subtype, of which the more common subtypes have been described earlier (Teige 1985, 1986). The Hp 3 (Johnson) has a 3 band pattern with one major and two minor bands. These bands are seen between the 2FS main band and the β- chain, Fig. 1, lanes 1 and 2. The Hp 2FF has a main band which are just a little more acidic than the β-chain, Fig. 1, lane 3.

HP 2-variant
The variant was observed in a blood sample from a suspect in a case of burglary. The Hp 2-variant has a slightly more acidic isoelectric point than 2FS, Fig. 1, lanes 4 and 5. This is most clearly seen in Fig. 4, lane 4, which shows a mixture of normal 2FS and the 2-variant. Two-dimensional SDS electrophoresis confirmed the variant's isoelectric point, and showed that the variant has the same molecular weight as normal 2FS.

Hp 1S-variant
This variant was observed in a child and in the alleged father. The alleged father is typed as 2FS1Svar, the child as 1S1Svar and the mother as 2FS1S, Fig. 1, lanes 6, 7 and 8. The variant's isofocusing pattern shows two bands of equal, but rather low

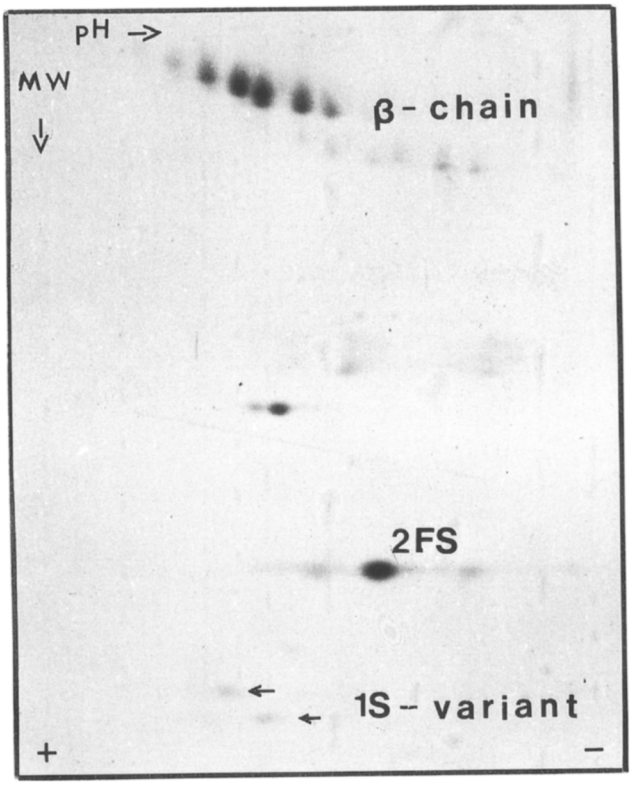

Figure 2.
Two-dimensional SDS electrophoresis of serum haptoglobin immunoprecipitate from alleged father showing the 1S-variant. The alleged father is typed as 2FS1Svar.

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intensity. One band has the same isoelectric point as normal 1S main band. The other band is more acidic, but a little less than the normal 1S minor band. The two-dimensional pattern of the variant shows two faint spots, fig. 2. One of the spots corresponds to normal 1S both in molecular weight and isoelectric point, but not in relative intensity to 2FS. The other spot is more acidic and heavier. As in the one-dimensional picture, the intensity of the two spots is equal.

Acid Hp 1-variant
An acidic α-chain variant is observed in a child and in the mother`s husband. The haptoglobin isofocusing pattern in serum from the child is 2FS1var., Fig. 1, lane 9 , and in serum from the husband 1S1var., Fig. 1, lane 10. The variant's main band has the same isoelectric point as the acidic minor band of 1S, and the variant's minor band is correspondingly more acidic. Two-dimensional SDS electrophoresis of the variant confirms its isoelectric point, and shows that the variant alpha peptide has the same molecular weight as 1F and 1S.

Phenotype distribution and allele frequencies
The haptoglobin phenotype distribution in 3318 unrelated individuals examined at the Institute of Forensic Medicine in Oslo, is presented in table 1. The allele frequencies determined in this material and in an earlier Norwegian material, are presented in table 2. Haptoglobin allele frequencies in Denmark, France and GDR are included in the table for comparison.

Hp-types	Observed	Expected
1S	183	170.0
1F	79	85.0
1F1S	240	240.4
2FS	1076	1086.4
2FS2SS	156	140.0
2FS2FF	16	14.4
2SS2FF	1	0.9
2SS	4	4.5
2FF	0	0.05
2FS1S	841	859.6
2FS1F	628	607.8
2SS1S	50	55.4
2SS1F	30	39.2
2FF1S	3	5.7
2FF1F	5	4.0
3(Johnson)	0	0.001
32FS	2	2.3
32SS	0	0.2
32FF	0	0.02
31S	1	0.9
31F	1	0.6
2FS1Svar	1	0.6
1S1Svar	1	0.2
N	3318	3318.2

Table 1. Haptoglobin (HPA) phenotype distribution in 3318 unrelated individuals involved in cases of disputed paternity.

$chi^2 = 7.528$
d.f.=10
0.70 >P >0.50
(Phenotypes with numbers less than 6 are counted together)

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The β-chain variant described earlier (Teige 1986) appears with a slightly higher frequency than Hp 3 (Johnson). We have seen the variant in 5 unrelated individuals, and also in mother and child.

Paternity exclusion efficiency

The paternity exclusion efficiency of the system is evaluated in a material of 507 men involved in paternity cases and excluded in other systems than haptoglobin. Of these 507 non-fathers 163 were also excluded by haptoglobin subtyping, which is in agreement with the expected exclusion efficiency of the system, table 3.

The total exclusion efficiency of the other routine systems used in cases of disputed paternity in our laboratory is approximately 92 percent, and the 507 excluded men therefore represent only 92 percent of the non-fathers in our material. Thus, 14 single exclusions with the haptoglobin subtyping system is expected, which is in very good agreement with the 18 observed.

Table 2. Haptoglobin allele frequencies in 3318 unrelated individuals involved in cases of disputed paternity

HP ALLELE FREQUENCIES	1F	1S	2FS	2SS	2FF	3(J)	
Shibata 1982 (France)	0.139	0.245	0.547	0.045	0.012		(N=202)
Thymann 1977 (Denmark)	0.16	0.27	0.54	0.03	0.002		(N=208)
Thymann 1987 (Denmark)	0.170	0.247	0.558	0.025	n.d.		(N=377)
Patzelt 1985 (GDR)	0.1471	0.2502	0.5753	0.0250	0.0020	0.0004	(N=1275)
Teige 1986 (Norway)	0.162	0.209	0.588	0.038	0.003		(N=606)
This material	0.160	0.226	0.572	0.037	0.004	0.0006	(N=3318)

n.d.= not determined

Table 3. Haptoglobin paternity exclusion efficiency in a material of 507 non-fathers[1]

	Observed	Expected
Number of Hp exclusions	163	165
Percent Hp exclusions	32.1	32.6

[1]Excluded in other systems

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DISCUSSION

The haptoglobin subtyping system has proved a reliable and efficient method for use in cases of disputed paternity. Only small amounts of serum is needed, and typing is achieved even in samples subjected to a high degree of haemolysis. The sensitivity of the method is very dependent on the antibody quality, and testing of antibody preparations, including different batches, from different commercial sources has been necessary. The antibodies presently in use in our laboratory have worked satisfactory for a long period of time.

The observed α 2-variant and the acid α 1-variant differ from normal 1 and 2 in isoelectric points only, and these variantions may be explained by single point mutations leading to an amino acid change in the peptides. The mechanisms behind the 1S-variant showing two peptides with different molecular weight, are not that easily explained. Further studies, preferrably on DNA level, might give informations on the genetic events leading to this variant.

The haptoglobin phenotype distribution found in the present material corresponds to Hardy Weinberg expectations, and the haptoglobin allele frequencies found in Norway are quite similar to the frequencies reported from other European countries.

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