# Haptoglobin (Hp) subtypes in the German Rhine-Ruhr area

J. Bertrams, B. Müller, W. Luboldt*

Prof. Dr. J. Bertrams, Abteilung für Laboratoriumsmedizin.
Elisabeth Krankenhaus, Moltkestr. 61, D-4300 Essen 1/FRG
*Blutspende und Transfusionsdienst, Universität Essen/GHS,
Hufelandstr. 55, D-4300 Essen 1/FRG

## INTRODUCTION

The two allelic polymorphism of the haptoglobin (Hp) system was detected by means of starch gel electrophoresis (SGE) more than 30 years ago (Smithies 1955). With an exclusion chance of 18% the Hp system has become a regular part of paternity testing since many years. Although Hp subtypes were discovered already in 1962 by Smithies et al., technical problems in producing unequivocal Hp pherograms of all five subtypes Hp1F,1S,2FF,2FS and 2SS hindered its common use in paternity testing. Only after application of isoelectric focusing (IEF) technique and after description of practicable techniques for purification of the Hp molecule (Oleisen et al. 1981, Santoro et al. 1982, Shibata et al. 1982, Patzelt and Schröder 1985) and after application of immunoblotting to Hp subtyping as well (Teige et al. 1983, 1986) all conditions for large scale routine Hp subtyping are given.

We present here Hp subtype frequencies determined in a large population from Western Germany (Rhine-Ruhr area) according to the method published by Patzelt and Schröder (1985), who worked out the technique of IEF of haptoglobin cleavage products. This method was developed by Shibata et al. (1982) and meanwhile investigated also by Zischler et al. (1987) in a Southwestern German population.

## GENES AND PROTEINS

A short comment should be given to the rare and interesting phenomena of duplication of genes as well as confirmation and polymerization of gene products existing in the Hp system. The Hp molecules are composed of two kinds of polypeptide chains, $\alpha$ and ß chains. The individual differences (genetic polymorphism) are based on variations in the $\alpha$ chain, with the ß chain being everywhere the same. Three alleles at the Hp locus on chromosome 16 are responsible for three main kinds of $\alpha$ chains in all mammals. Two of the alleles, Hp*1F and Hp*1S, control $\alpha$ chains that differ from each other only by a single specific replacement of one amino acid ($\alpha$1 chains). The third allele, Hp*2, differs from both Hp*1 alleles in a much more striking way. It is nearly twice as long as the Hp*1 gene and its gene product ($\alpha$2 chain) shows to be a combination of two nearly complete $\alpha$1 chains arranged in tandem sequence. The formation of the Hp*2 allele obviously was the result of a genetic "accident" (Smithies et al. 1962, Nance and Smithies 1962), in which an ancestral Hp gene of normal length underwent an unequal crossing over with its allele in the homologous chromosome but at a non homologous site. Since two Hp*1 alleles exist, Hp*1S and Hp*1F, three different Hp*2 alleles

Advances in Forensic Haemogenetics 2
Edited by W.R. Mayr
© Springer-Verlag Berlin Heidelberg 1988

resulted from the unequal crossing over, Hp*2FF, *2FS and *2SS. Accordingly altogether five Hp alleles can be identified by IEF techniques.

The confirmation and polymerization of Hp gene products ($\alpha 1$, $\alpha 2$ and ß chains) is another point which has to be explained for a better understanding of the Hp system. After transcription and processing the Hp m-RNA initiats the translation of one large polypeptide chain, which contains the $\alpha$ and ß parts of the Hp molecule. These chains are cleaved by limited proteolysis to form an $\alpha$-ß dimer. An individual, being homozygous for the Hp*1 gene, forms tetrameric molecules with two ß chains and two $\alpha 1$ chains. According to the alleles Hp*1F and *1S, both $\alpha 1$ chains carry the same or both allotypes. An individual being homozygous for the duplicated H*2 gene produces dimers with the larger $\alpha 2$ chains. Each of these chains carry Hp subtypes according to the composition of the Hp*1 genes (1F,1S), which had produced the Hp*2 gene by unequal crossing over, Hp*2FF, *2FS or *2SS. An individual being heterozygous for the Hp*1 and Hp*2 genes forms tetramers out of different dimers with short $\alpha 1$ and long $\alpha 2$ chains. According to free cysteine residues of the $\alpha 2$ chains Hp2 homozygote and Hp 2-1 heterozygote molecules tend to polymerize according to following formulas. Hp 2-2: $(\alpha 2ß) \cdot n$, n= 3,4,5...; Hp 2-1: $(\alpha 1ß) \cdot 2x (\alpha 2ß) \cdot n$, n=0,1,2...Multiple Hp bands seen in SGE are indicators of different degress of polymerization (Yang and Prybylska 1983, Pastewka et al. 1985).

MATERIAL AND METHODS*

Sera
Non hemolytic sera from 1035 normal, unrelated Caucasian blood donors of the German Rhine-Ruhr area.

Hp purification
100(200µl) of non hemolytic serum was added to 2ml(4ml) DEAE suspension which was prepared by mixing one part of a 1% aqueous DEAE stock solution with two parts of a 10mM sodium acetic buffer (pH 4.7). After centrifugation at 5000g for 5min. the clear supernatant was sucked off. To elute the hemoglobin moleculesthe pellet was washed with 2ml of the sodium acetic buffer and resuspended with 100µl (200µl) of a 0.125M ammonium acetic solution. After additional centrifugation for 10min. 40µl of the clear haptoglobin containing supernatant was transfered to a second tube for reductive cleavage.

Reductive cleavage
40µl Hp containing supernatant was mixed with 40µl of a reductive reagent, which was prepared by dissolution of 1g urea in 1.2ml borate buffer of pH 8.8 (0.1M boric acid, 0.04M NaOH) plus 20µl of ß-mercaptoaethanol. The mixture was incubated for 30min. and 8µl of an iodacetamide solution (92mg iodacetamide in 1ml of aqua dest.) was added to prevent refolding of the separated $\alpha$ and ß chains of the Hp molecule.

Isoelectric focusing and staining
IEF of the cleavage products was performed in polyacrylamide gels (T=5.5%, C=3%; 260x125x0,5mm) on gel bond films using the LKB

*A very detailed description of the method is availabe at request.

equipment (Ultrophor, Macrodrive 5, Multitemp II). Before polymerization an ampholine mixture (Ampholine LKB, two parts pH 3.5-10, two parts pH 5-7. one part pH 6-8) is added in 5% proportion of the total gel (0.8ml carrier ampholytes for a 16ml gel). Gels can be stored at 4-6°C up to a fortnight. Electrode paper strips for prefocusing and focusing were soaked with 0.5M NaOH for the cathode and with 0.5M $H_3PO_4$ for the anode. After prefocusing of the gel for 30min. at 5-10°C samples were applicated on to the gel by applicator pieces (Whatman No1, 5x10mm). IEF was performed for 150min. Applicator pieces were revomed after 30min. Maximum electric values for prefocusing and focusing were 1600V, 10mA and 10W. Fixation, staining and decolorization of the gels were performed according to Steck et al. (1980).

RESULTS

Hp subtype patterns obtained by IEF of purificated and cleaved Hp proteins are presented in Fig.1. According to an isoelectric point of pI=6.52 the major band of Hp 2FF appears in our technique in the very cathodal region of the gel near the sample application area.

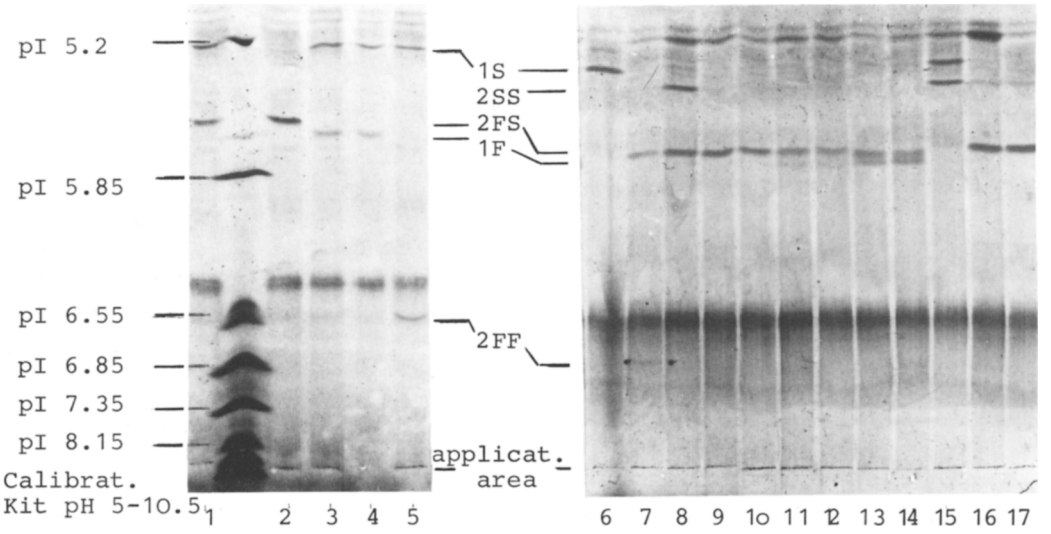


Fig. 1. Hp subtypes from left to right: 1) 1S-2FS 2) 2FS 3) 1S-1F 4) 1S-1F 5) 1S-2FF 6) 1S 7)2FS-2FF 8)2SS-2FS 9) 2FS 10) 2FS 11) 2FS 12) 2FS 13) 2FS-1F 14) 2FS-1F 15) 1S-2SS 16) 2FS 17) 2FS

Observed and exptected phenotype frequencies and the corresponding gene frequencies of Hp subtypes are presented in Table 1. There is no deviation of the observed from the expected values.

Comparison with allele frequencies obtained by other investigators including different techniques also yielded very good aggrement (Table 2).

Table 1. Hp phenotype distribution among 1035 unrelated individuals and corresponding gene frequencies

| Phenotype | Observed n | Observed % | Expected n | $\chi^2$ | allele frequencies |
|---|---|---|---|---|---|
| 1F -1F | 19 | 1.84% | 20.03 | 0.05 | |
| 1F -1S | 74 | 7.15% | 74.16 | 0.00 | Hp*1F = 0.1391 |
| 1S -1S | 69 | 6.67% | 68.62 | 0.00 | *1S = 0.2575 |
| 1F -2FF | 0 | 0.00% | 0.42 | 0.42 | *2FS = 0.5831 |
| 1F -2FS | 172 | 16.62% | 167.93 | 0.10 | *2SS = 0.0188 |
| 1F -2SS | 4 | 0.39% | 5.43 | 0.37 | *2FF = 0.0014 |
| 1S -2FF | 0 | 0.00% | 0.77 | 0.77 | |
| 1S -2FS | 309 | 29.86% | 310.79 | 0.01 | |
| 1S -2SS | 12 | 1.16% | 10.04 | 0.38 | |
| 2FF-2FF | 0 | 0.00% | 0.00 | 0.00 | |
| 2FF-2FS | 3 | 0.29% | 1.75 | 0.89 | |
| 2FF-2SS | 0 | 0.00% | 0.06 | 0.06 | |
| 2FS-2FS | 350 | 33.82% | 351.90 | 0.01 | |
| 2FS-2SS | 23 | 2.22% | 22.74 | 0.00 | |
| 2SS-2SS | 0 | 0.00% | 0.37 | 0.37 | |

Sum of Chi-square for (df 10)=3.44 (0.975>p>0.95)
(df 5)=0.97 (0.975>p>0.95)

Table 2.Hp allele frequencies of different studies

| Study | n | Hp alleles *1F | *1S | *2FS | *2SS | *2FS |
|---|---|---|---|---|---|---|
| Bertrams et al.1987 | 1035 | 0.1387 | 0.2538 | 0.5864 | 0.0196 | 0.0015 |
| Thymann et al.1977 | 208 | 0.16 | 0.27 | 0.54 | 0.03 | 0.00 |
| Oleisen et al.1981 | 52 | 0.13 | 0.21 | 0.63 | 0.03 | 0.00 |
| Shibata et al.1982 | 202 | 0.1421 | 0.2462 | 0.5533 | 0.0457 | 0.0127 |
| Patzelt and Schröder 1986 | 1275 | 0.1471 | 0.2502 | 0.5753 | 0.0251 | 0.0020 |
| Teige et al. 1986 | 606 | 0.162 | 0.209 | 0.588 | 0.038 | 0.003 |
| Zischler et al.1987 | 182 | 0.144 | 0.254 | 0.574 | 0.024 | 0.0004 |

DISCUSSION

The excellent agreement of Hp allele frequencies obtained by different groups especially among the same population demonstrates the usefulness of Hp subtyping for paternity testing. No single deviation for expected Hp subtypes according to results obtained by SGE was observed among more than 200 matings. The exclusion chance of the Hp system increases from 18% by SGE to 33% by IEF. This is the highest exclusion chance of all IEF systems as shown in Table 3.

Table 3. Exclusion chance of IEF systems(Mendner and Kühnl 1986)

| System | Exclusion chance | System | Exclusion chance |
|---|---|---|---|
| Hp | 33% | C8 | 19.27% |
| PGM1 | 31.91% | C6 | 18.77% |
| GC | 29.74% | A2HS | 17.81% |
| PI | 29.29% | FUCA | 15.10% |
| GDH | 26.90% | ESD | 9.77% |
| PLG | 22.58% | AMY2 | 4.52% |
| F13B | 22.29% | C2 | 2.83% |
| TF | 19.43% | | |

References

Mendner TT, Kühnl P (1986) Alpha-2-HS Glykoprotein polymorphismus. In: Brinkmann B, Henningsen K (eds). Advances in Forensic Haemogenetics 1, p 167

Nance WE, Smithies O (1962) New haptoglobin alleles: A prediction confirmed. Nature 198: 869-870

Olaisen B, Mevaag B, Teige P, Gedde-Dahl T, Holland R, Siverts A, Jonassen R, Hjalmarsson K, Swan T (1981) Plasma protein polymorphisms in two-dimensional electrofocusing/SDS electrophoresis. Proc. 9th International Congress of the Society Forensic Haemogenetics, p 543

Pastewko JV, Reed RA, Ness AT, Peacock AC (1973) An improved haptoglobin subtyping procedure using polyacrylamide gel electrophoresis 51: 152-162

Patzelt D, Schröder H (1985) Haptoglobin subtypes in Berlin/GDR. A simple procedure for haptoglobin purification and subtyping, Z Rechtsmedizin 94: 207-212

Sontoro C, Boccazzi C, Corbonara AO (1982) A simplified procedure for haptoglobin subtyping. Hum Genet 60: 287-288

Shibata K, Constans J, Viau M, Matsumoto H (1982) Polymorphism of the haptoglobin peptides in isoelectric focusing electrophoresis and isoelectric point determinations Hum Genet 61: 210-214

Smithies O (1955) Zone electrophoresis in starch gels, group variations in the serum proteins of normal human adults. Biochem J 61: 629-641

Smithies O, Connell GE, Dixon GA (1962) Inheritance of haptoglobin subtypes. Am J Hum Genet 14: 14-21

Steck G, Leuthard D, Bürk RR (1980) Detection of basis proteins and low molecular weight peptides in polyacrylamide gels by formaldehyde fixation. Anal Biochem 107: 21-24

Teige B, Mevag B, Olaisen B, Pedersen L (1983) Haptoglobin subtyping in routine paternity cases. Presentation of a promising new method. Proc. 10th International Congress of the Society of Forensic Haemogenetics, p 136

Teige B, Olaisen B, Pedersen L (1986) Subtyping of haptoglobin. In: Brinkmann B, Henningsen K (eds). Advances in Forensic Haemogenetics 1,p 136

Thymann M. (1977) Improved haptoglobin subtyping demonstration in the Danish population. Proc 7th International Congress of the Society for Forensic Haemogenetics, p 373

Yang HJ, Prybylska M (1973) The microheterogeneity of human haptoglobin and its complex with haemoglobin Can J Biochem 51, 597-605

Zischler H, Kömpf J, Ritter H (1978) IEF of Hp-subtypes: presentation of population genetic data and variants. Ärztl Lab 33: 85-87